CARNICOM INSTITUTE LEGACY PROJECT

A Release of Internal Original Research Documents

Authored
by
Clifford E Carnicom
President, Carnicom Institute

Laboratory Notes Series: Volume 19
Jun 2017 – Jul 2017

www.carnicominstitute.org
www.wikici.org

Carnicom Institute is a non-profit organization, 501(c)(3), working solely for the benefit of humanity and the public interest. Our goal is to provide the public with beneficial and responsible information concerning human health and the environment. The Institute is extensively active in conducting scientific research and public education relating to the consequences of geoengineering and bioengineering. Thank you for your support of Carnicom Institute.

TOP | FLIGHT

CHEMISTRY VOL XIX

www.topflightpaper.com

SUSTAINABLE FORESTRY INITIATIVE Certified Sourcing www.sfiprogram.org SFI-00931

0 75755 31804 5

3 SUBJECT
3 À SUJETS

120 SHEETS/ FEUILLES

Chemistry - Lab Notes

Jun 2017

Vol XIX

Jun 14 2017 (cont)

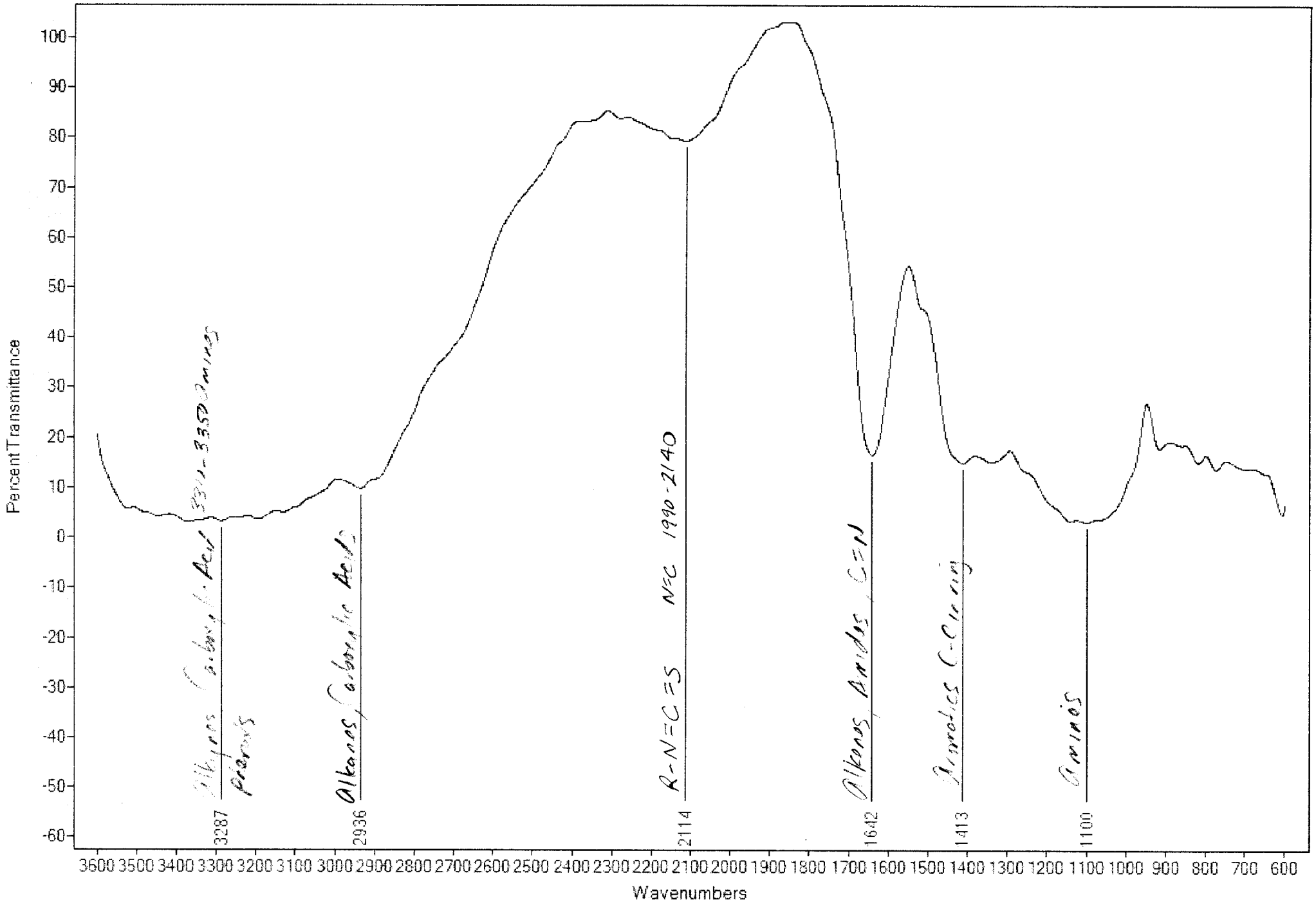
We are going to distill the COB secreted protein in an attempt to assess the water content.
Sample volume is ~27 ml. extracted from 3 tubes.

mins	secs		
		First boiling point 97.7°C = 98°C ≅ H_2O.	
1	15	97.8°C	There is some odor however.
3	15	97.9°C	
8	15	97.9	Odor diminishing.
11	30	98.1	
17	45	98.1	
22	30	98.1	Vol ≅ 8 ml
25	00	98.1	Boiling appears to be changing (15 ml)
29	00	98.0	Vol ~ 7 ml.
31	15	97.9	End

Approx 9 ml left in beaker.
Milk colored solution of density is sufficient.

We do indeed have a very concentrated form of the protein remaining from the distillation procedure. Approx 4 ml have been captured. Also a 100 ul highly concentrated IR sample has been run.

CDB Concentrated Secreted Protein by Distillation Jun 14 2017 - 02.JPEG



Jan 15 2017 Protein Size, Gene Size

Maya unites w/ digital thermometers today.
3 out of 4 digital thermometers have failed.
In a backup mode - the glycol Omnitherm has accurate temperature logging within it (Handles up to 1200°C!)

It would be good to get good thermometers.
4 general thermometers on order.

Next, we are quite certain the Concentrated CDB secreted protein has been denatured. This is evident from significant color change, now a deep amber color, and also the milky appearance occurring during distillation.
Lets adjust our molecular weight for the presence of water.

Original sample is ~27 ml. MW estimated @ 1276 gm/mol but this assumes a pure sample.

We now know that approx 18 ml of the sample was H_2O.
ie. 2/3 was water, now assume 1/3 is protein.

We now therefore increase the MW estimate by a factor of 3.

MW Estimate for CDB secreted protein is

now: 1276 (3) = 3828 gms/mole

a MW ≅ 3828 daltons or ~ 3.83 KdA
≅ 4 KdA

What other proteins are in the size class?

The smallest protein known, which is derived, ie artificial from the saliva of Gila monsters. (~20 amino acids)

An amino acid is about 100 dA, this would lead to a size of approx 20(100) = 2000 dA or ~ 2 KdA.

1 dA = $\frac{1 gm}{mol}$ & 1 KdA = $\frac{1000 gms}{mol}$.

Our protein estimate is ~ $\frac{3828 gms}{mol}$ or ~3828 dA
or ~ 3.8 KdA. This would lead to an amino acid sequence of approx $\frac{3828 dA}{100 daltons/amino acid}$ ≅ 38 amino acids in the chain.
The smallest human protein is 44 amino acids so there is on par w/ that.

There is indeed a relationship established to predict the gene size based upon the protein size. It comes from Univ of Pittsburgh & a Russian site.

The estimate given as that
Protein Size ≅ Gene Size
4 KDa ≅ 0.108 kb

Page 5

Published on *Science 2.0* (http://www.science20.com)

The smallest protein

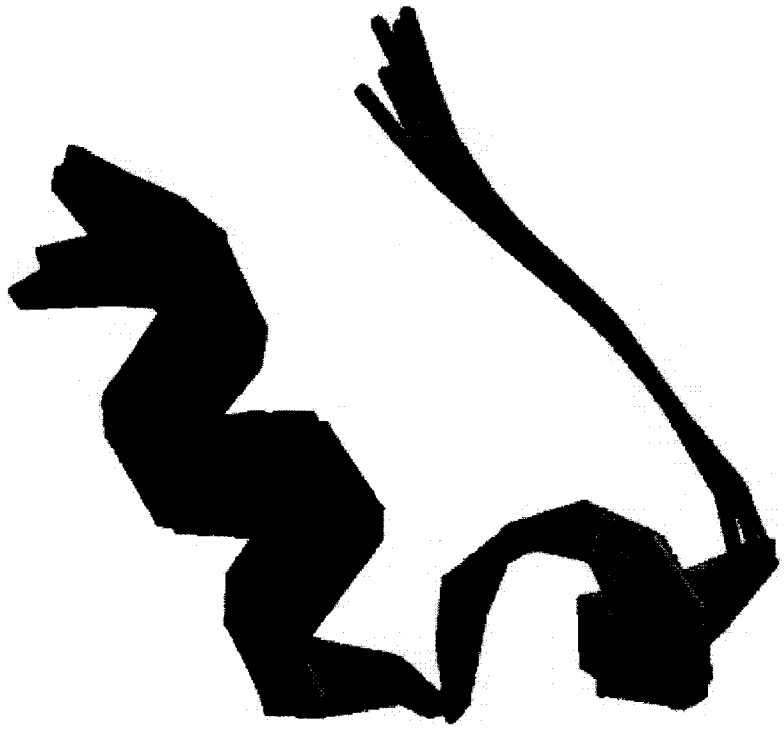
By *Yu Zhang*
Created *Oct 9 2010 - 10:32am*

Ever wonder what the smallest protein is? Apparently it's TRP-Cage, a protein with only 20 amino acids derived from the saliva of Gila monsters.

Trp-cage - smallest protein

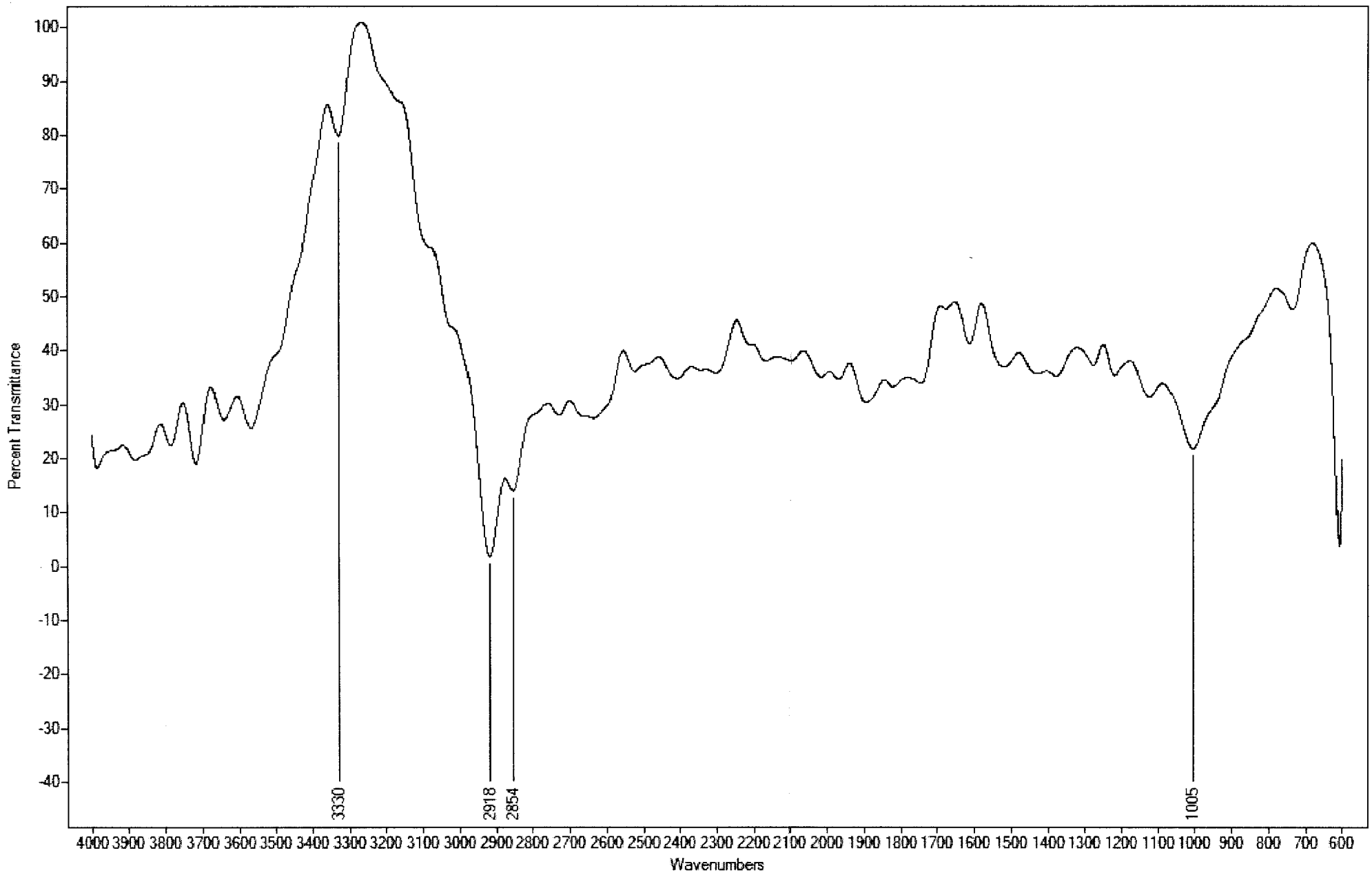
You can find the structure file and images in the PDB database (www.pdb.org) with PDB ID = 1L2Y. This highly stable mini-protein is important for studies of protein stability, protein folding, and 3D structure.

Even with this small size, it displays secondary structural elements, such as an alpha helix, found in many proteins. So far there are no known proteins with less than 20 residues, but we'll see what happens in the future.



Genetics & Molecular Biology

Rainfall Concentrate Residfual ATR Gain 01 Jun 16 2016 - 11_ras.spc: 06/17/2016 02:39:10 title



printed on 06/17/2016 02:51:57

Page 5 B

Ho

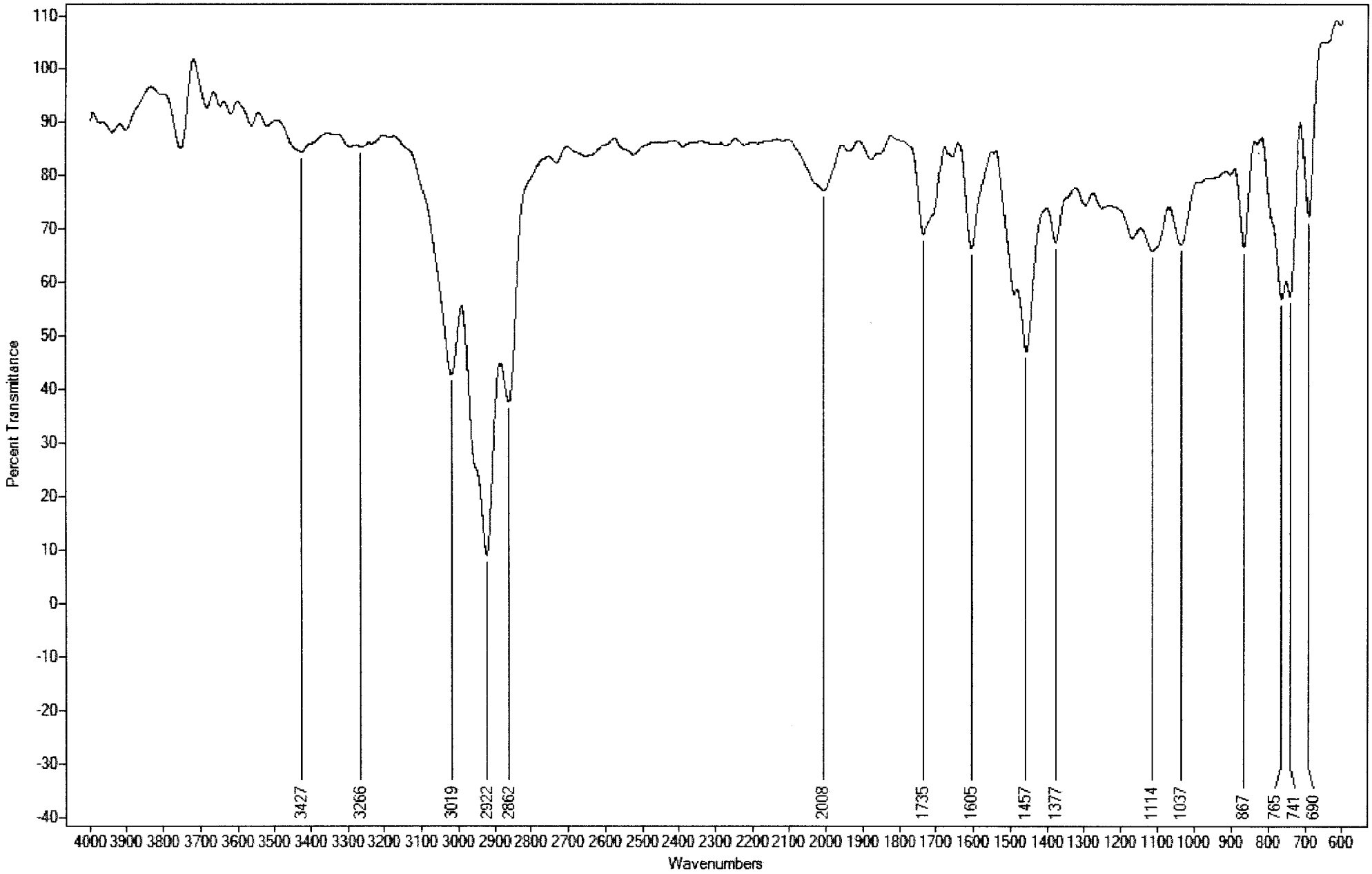
DNA Size (kb) Protein Size (kDa) Conversion Tool

URL:	http://www.molbiol.ru/eng/scripts/01_06.html
What you can do:	Estimate the size of the gene (kb) by the protein size (kDa) and vice versa.
Highlights:	• Calculate the gene size from the protein size and vice versa.
Keywords:	• gene size • protein size • lab tools
Literature & Tutorials:	

This record last updated: 05-06-2005

CDb Lipids 208 Degrees May 30 2016 - 02_trn.spc: 05/31/2016 01:53:32 title

Page 5C



printed on 05/31/2016 01:59:16

Page 50

See also:
/web-links/

Extended form Close/Open

DNA to Protein

This program helps you to estimate the size of the gene by the protein size and vice versa: the size of the protein by the size of the gene.

Reset

DNA --> protein:

1 [kb] Convert [kDa] AA

Protein --> DNA:

4 [kDa] Convert 0.108 [kb]

Sequences may be translated with programs: "Six-Frame Translation" and "Reverse translation of aminoacid sequences". For manipulations with nucleic acid sequence (reverse, reverse/complement, double stranded) it is possible to use Sequence Utilities program.

Zbio.net: http://zbio.net
e-mail: editor@zbio.net
seen: 69207

Supplement

There appears to be an error with the database.
You can try to refresh the page by clicking here.

Error Returned

```
mySQL query error: SELECT permission_custom_error FROM
ipb_forums WHERE id=

mySQL error: You have an error in your SQL syntax; check the
manual that corresponds to your MySQL server version for the
right syntax to use near '' at line 1
mySQL error code:
Date: Thursday 15th 2017f June 2017 07:22:17 PM
```

We apologise for any inconvenience

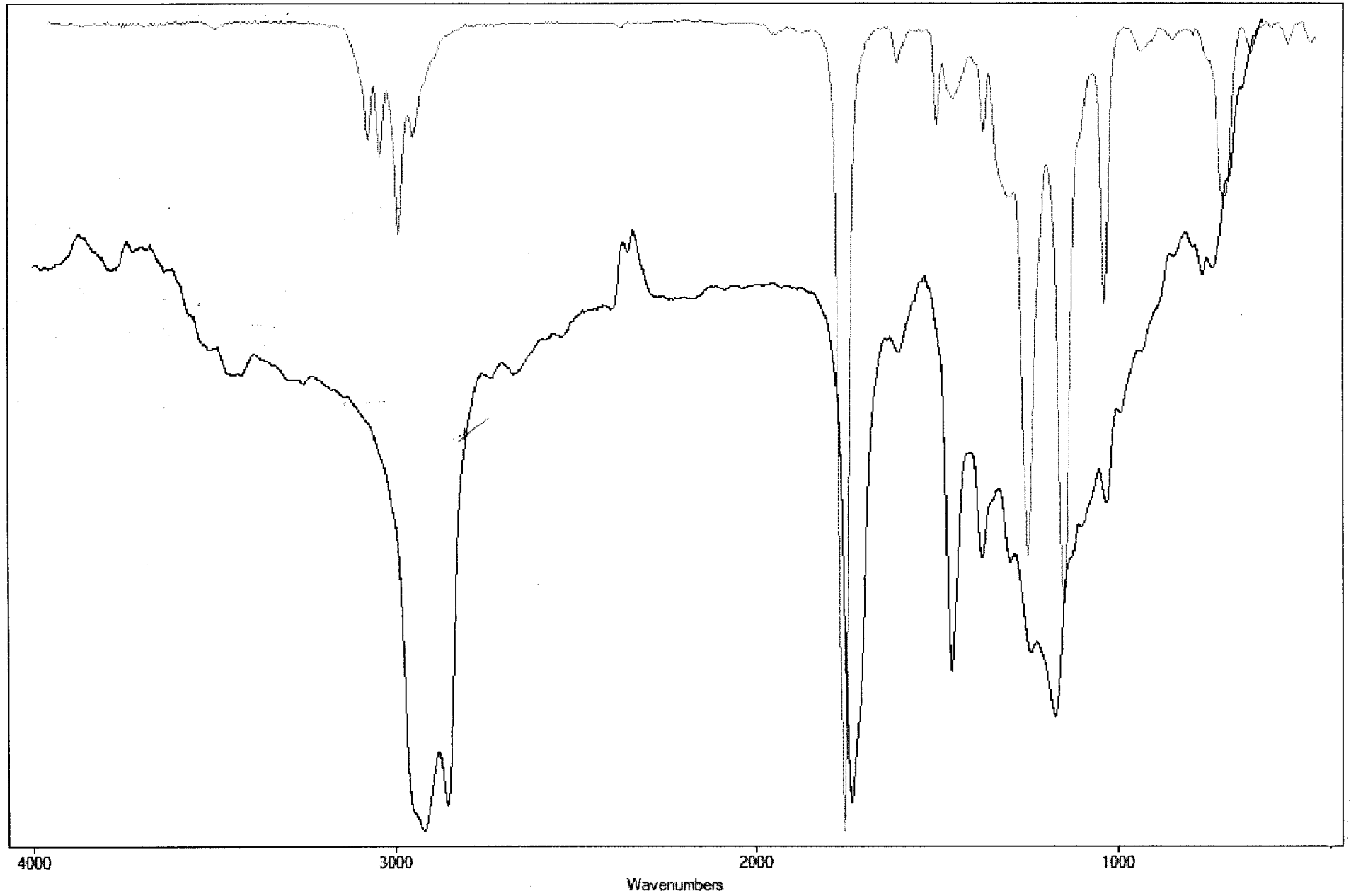
--- страница форума с комментариями --

Extended form Hide/Show

Steve Schafer, Coder, part-time physicist, birder
Answered May 13, 2015

The smallest known protein, Trp-cage, contains 20 amino acids, so it has, at minimum, a genetic code containing 60 base pairs. However, it is an artificially created protein, so it doesn't really count (it does, however, provide a lower bound on what's possible). There are also tRNA genes, which aren't genes in the normal sense, but rather instructions for constructing tRNAs. The shortest known tRNA gene is 76 base pairs. (I don't think tRNA genes really count, either.)

The shortest known *functional* gene (that is, it codes for a protein that is actually used by the organism) is a histone gene that is about 500 base pairs long. Histones are proteins that are involved in organizing DNA into *nucleosomes*, which are the compact basic building blocks of chromosomes.


4000
3000
2000
1000
Wavenumbers

It is possible that we have an extremely small protein and a extremely small genome involved w/ the organism. Time will tell but these are the first indications.

Lets look @ IOR & UV data

Brix @ COB Secreted Concentrate is 29.9
This leads to an IOR of 1.379

The index of refraction for a protein is stated to be directly proportional to its concentration. 1934

Index of refraction is a unique characteristic of a protein. ACS 1964

UV: We should also look @ how the clarified Concentrate compares to the original.

I can see that it is extremely concentrated w/ max absorbance @ 280 nm — defiantly protein. The w/ no drop in cuvette absorbance is @ 2.95 and is @ an absolute max of λ_{max} 280.0. We look to be a really pure protein sample.

Peaks @ 280nm & 222 nm.

Ok, we have a superb sample which is highly Concentrated.

Page 7

Label go to Bradford, IR, and
Photograph the results

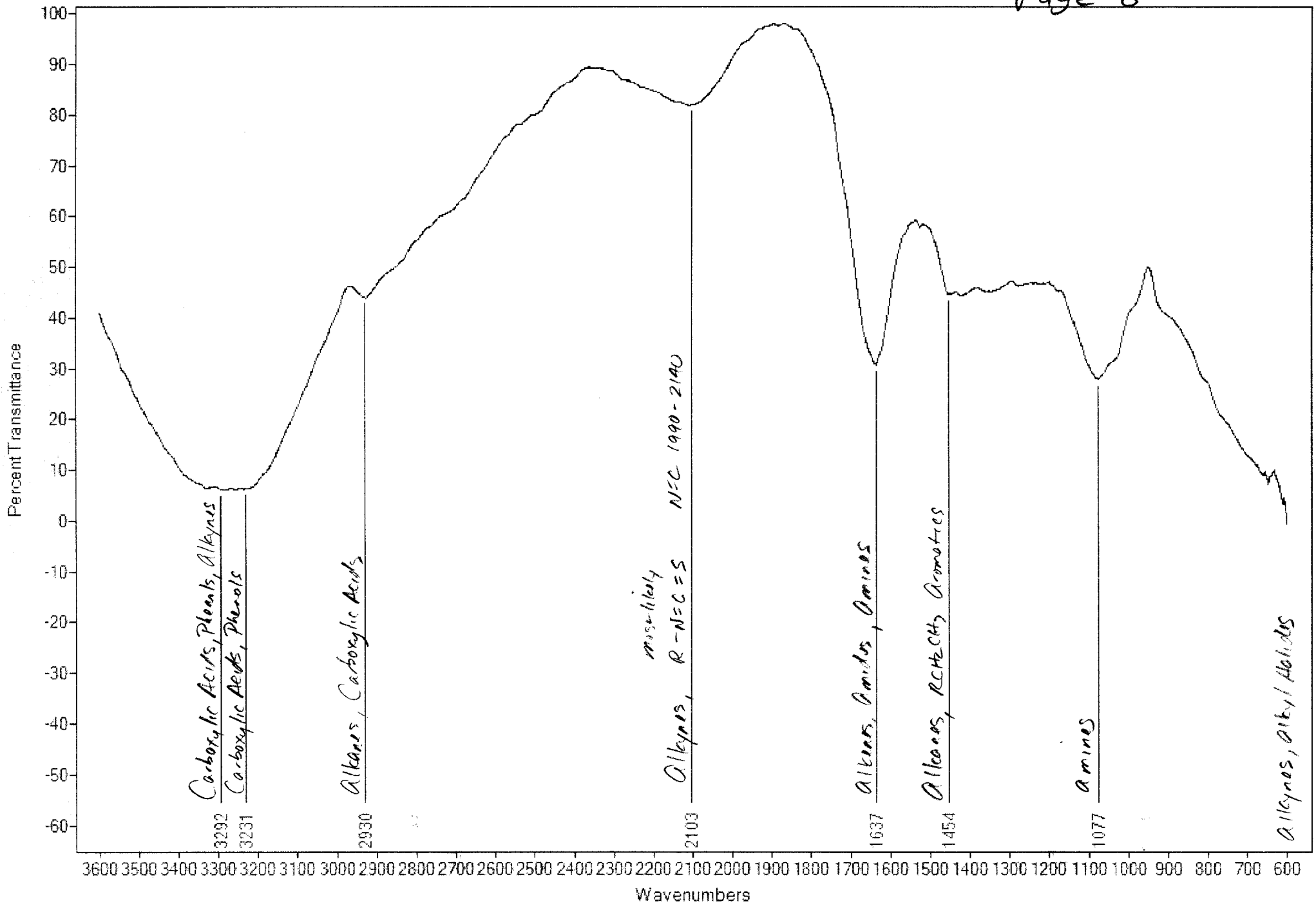
Bradford Test: Highly positive results.
Spectrum recorded & photograph taken.

IR: Protein Comparisons Required
Compare COB secreted distilled (denatured)
COB secreted original
HEPA and fulvic LC protein
Rainwater concentrate - protein
Original COB Protein Extraction

Five Proteins Nos 1 2 3 4 5

all water must be removed for all samples
salting out if required.

CDB Concentrated Secreted Protein by Distillation Synthesis of KCl and ATR Jun 15 2017 - 05.JPEG



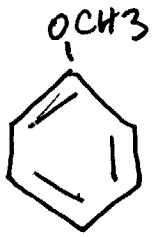
Let's work on the suspected ethyl acetate extraction
A perfectly representative problem to solve.

We have a white precipitate settled to the bottom of a tube w/ clear solution above it.
Approx total volume = 10 mL.

Let's first make a coarse separation not by hand.

The first thing that we learn is that the "clear" solution is hardly clear. There is a great deal of absorbance in UV.
Could it be partially dissolved ethyl acetate?
We have peaks @ 224 & ~262
Brix = 8.2 ⇒ IOR = 1.344

Anisole is one type of candidate



I do not have enough material for NMR exploration or likely post-examination

You would have to try for some functional groups.

I can call now the solution is definitely not water based.

You must try to get some functional group information first

—

I let the drying watch glass get too warm and all the solution evaporated. It did not mix w/ water so, it is not a polar solvent. We need new material.

Jan 16 2017

Working on the LC HEPA extract, opaque elute. Interestingly enough, IR analysis indicates the possible presence of amine/amides. Signals are @

3335] all of which tie into amine
1605] or amide.
1063] We also have alkane @ 2933.

The signal, however, is extremely weak and difficult to extract even upon concentration and evaporation.

We also learn that the precipitate does not form right away, it can not even be centrifuged to settle. Apparently it takes several days for the precipitate to form.

We need to accumulate the elute, continue to attempt to concentrate / evaporate to pull out IR signal. Also allow precipitate to form to work with it more directly. It does seem like the solvent of the elute is largely alcohol.

The Bradford test does come out completely negative however. In addition, however, the concentration appears so weak that Bradford may simply not be a reliable indicator here. I do believe we need the precipitate also.

What we see now is that the elute is only partially alcohol and the majority is water. The residue of evaporation is strongly damaging to the KCl crystal and it also absorbs IR very poorly. It is not very good form to work with.

The best course is to let the material precipitate out over several days and see if we can work w/ that material more directly. We may have some extremely weak protein that is dissolving here ahead of the primary protein (colored) that has been extracted along w/ a generally non absorbent IR immune crystal a precipitate. At some point, if time permits you have a clean HEPA air filter for control analysis. Time will be the factor on that one.

We now have a decent IR plot of the opaque elute from the HEPA air filter. It is difficult to capture but I have it.

There are definite signals for alkanes & amines. Also an aldehyde indicator strongly @ one point. This is therefore not ethyl acetate or an adulterant from the filter. It appears to be another aspect of the protein which elutes late (colored) when the column is subjected to strong alkaline.

The opaque forerunner elute w/ H_2O added to the column.

The signal is weak but it is positive.

Our constituents are expected to be

alkanes
amines
amide
potential aldehyde

Our peaks are @

3342	strong	amines
2940	moderate	alkanes
2865	weak	alkanes
1727	weak	aldehyde
1610	moderate	amide
1142	strong	amines
1054	strong	amines
~ 700	strong	amines

UV Peaks:

221 nm

272 only slightly detectable

We have gradually and steady increase in absorbance, esp from 290 to 220 nm. We have no real peak @ 280 nm and therefore no evidence of a protein along with failure of the Bradford test.

We know, therefore, now that the opaque eluate is not a protein. It appears to be primarily a combination of alkane and amines, with a potential aldehyde.

You would think that ninhydrin would pick up on the amines. They are weak but lets try it.

Do not use old ninhydrin solution. Recommend it be renewed upon any presence of color. Original ninhydrin in acetone is clear, aged ninhydrin in acetone is yellow.

We definitely have a reaction of ninhydrin with the dilute opaque eluted. Absorbance starts around 600nm and continues to increase all the way down to 400. They see it is starting to appear purple (which we could see vaguely by eye also) but it also continues into the appearance of yellow, as we also see with certain amino acids, also as it relates to pH.

Our control in this case was the dilute opaque eluate alone (15 drops in cuvette) prior to ninhydrin being added.

This would verify the existence of amines in the opaque eluate however there appears to be a separate precipitate that only slowly settles out in addition to the amine compounds.

Repeat Ninhydrin Test:

Yes, we have now verified the presence of an amine within the opaque HEPA elute. It is incredibly weak but nevertheless has been detected with an absorbance peak @ λ max = 573 nm

There is a mix of the middle of purple appearance and yellow seed absorbance.

We do have additional absorbance in the blue and violet sector (this is visible as yellow).

The entire process w/ IR & ninhydrin verification of amine presence has been difficult as it exists @ the threshold of detection but it has been verified on both accounts, IR & ninhydrin.

There is no known presence of a protein within the particular elute (opaque) but the presence of an amine compound has been verified.

The colored elute has been viewed as a protein and this will be done again & a careful comparison made with it to the CAB secreted protein.

Good work today @ the detection threshold.

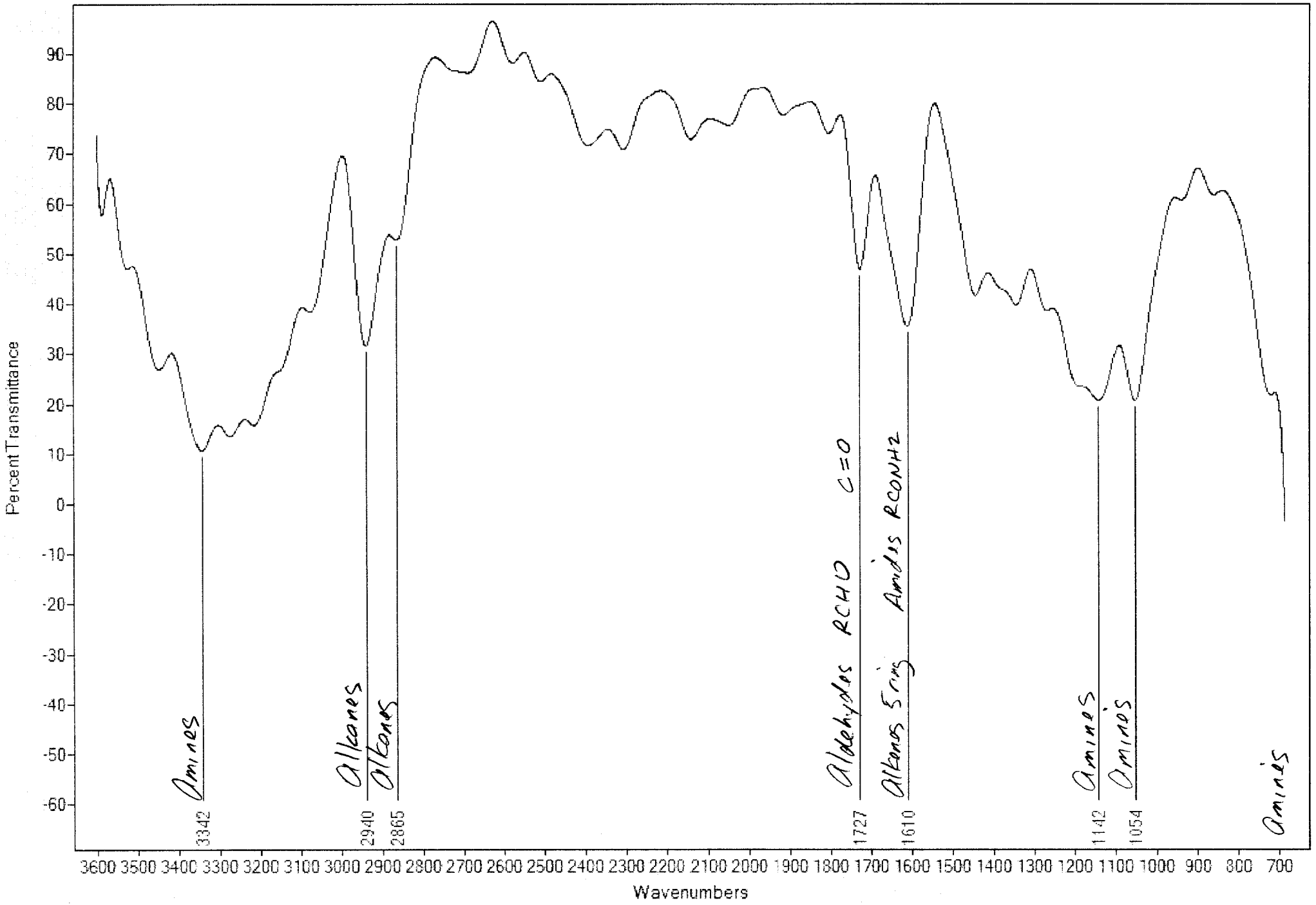
The Control in this case for UV was the dilute opaque eluate (10 drops in cuvette of H_2O) that was also heated in the water bath (but w/out ninhydrin added.

The actual sample under testing did have ninhydrin added (6 drops of 0.5 gms ninhydrin per 60 ml acetone).

Your eye is quite sensitive ~~to~~ as were able to pick up the purple tint. Absorbance magnitude, however, is only ~ 0.003 but it is clearly identifiable w/ the new UV instrument.

HEPA Filter Opaque Elute Jun 16 2017 - 01.JPEG

Page 16A



We want to learn the general properties of amines along the way here. Now that we have one.

Amines are "ubiquitous in biology".

Let's go back to LC2, the colored & verified protein. We have 2 versions of a protein to compare this to:

1. CBB stained protein
2. CBB stained but denatured protein as received from distillation

The protein undoubtedly has a lot of water in it and will need to be evaporated.

Lets start w/ UV and see if any absorbance can be picked up @ 280nm. We now have 2 40 ml tubes to work with.

5 drops of 1-8 tube into H_2O cuvette. We see two discontinuities (minor) w/ one of them @ ~ 273 nm but no real peak @ 280 nm for protein. Protein has been verified through Bradford.

1/3 Cuvette w/ H_2O
Discontinuity @ 340 nm from bulb switchover.

No single absorption peak. Steady increase from 300 – 230 nm, then sharp increase to 220 nm with no single peak.

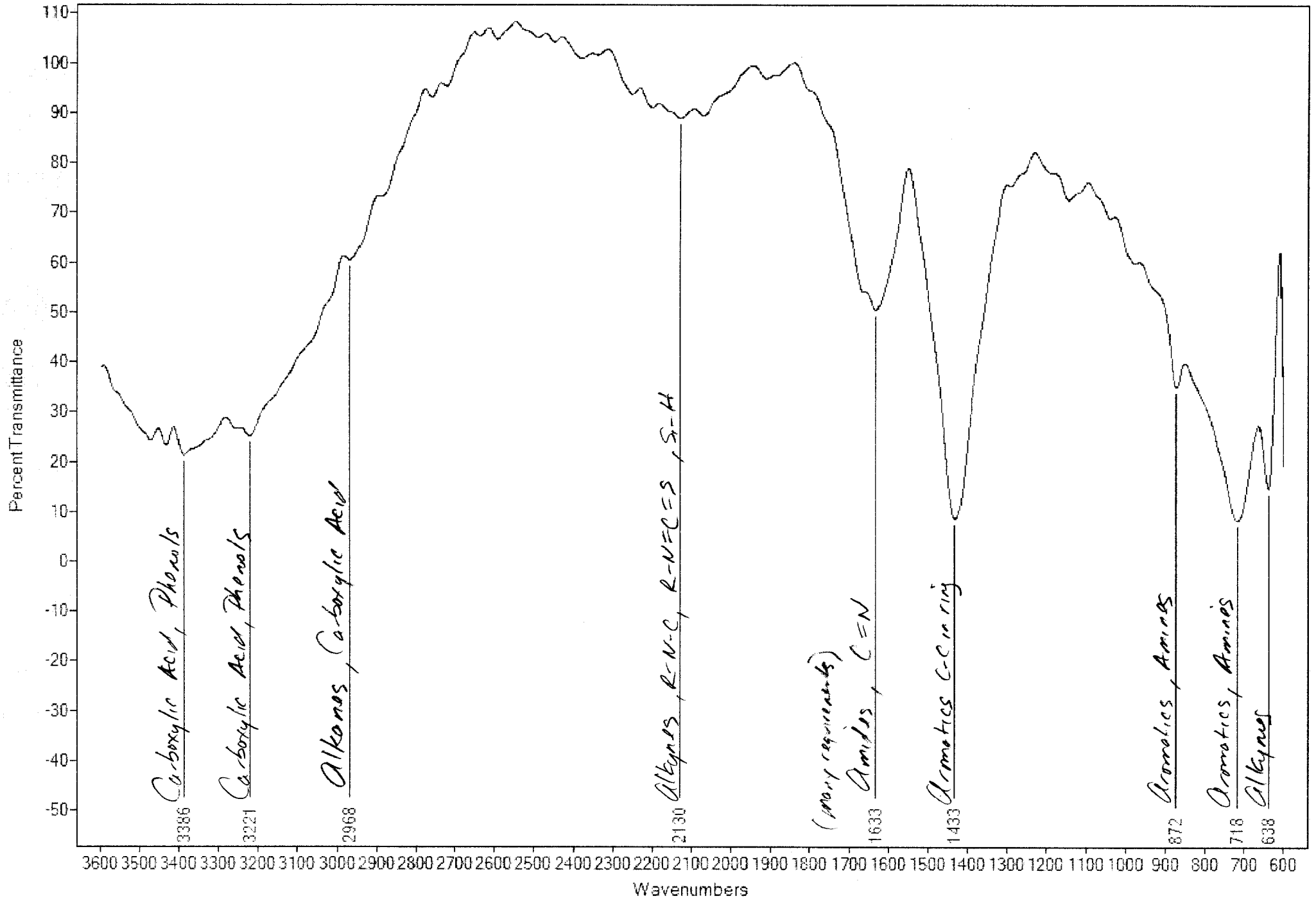
Now let's evaporate the sample, but before that Compare UV scan from tube 2 (1-1)

The plot is identical. Both tubes contain the same eluate. This is good in terms of consistency from separate runs.

Now evaporate about 10 ml.

We will also start a large scale anaerobic culture. ~250 ml H_2O, 1 tbsp sugar (sucrose), 1 tsp $FeSO_4$, ⅛ tsp salt ($NaCl$).
See notes of May 27, 2017. Vol 1B.
Call it LAN 1 / Large Anaerobic #1

HEPA Filter Colored Protein Jun 16 2017 - 01.JPEG



The UV absorbance of the secreted protein is essentially identical to that of LC #2, the colored protein. Both samples evaporated of water.

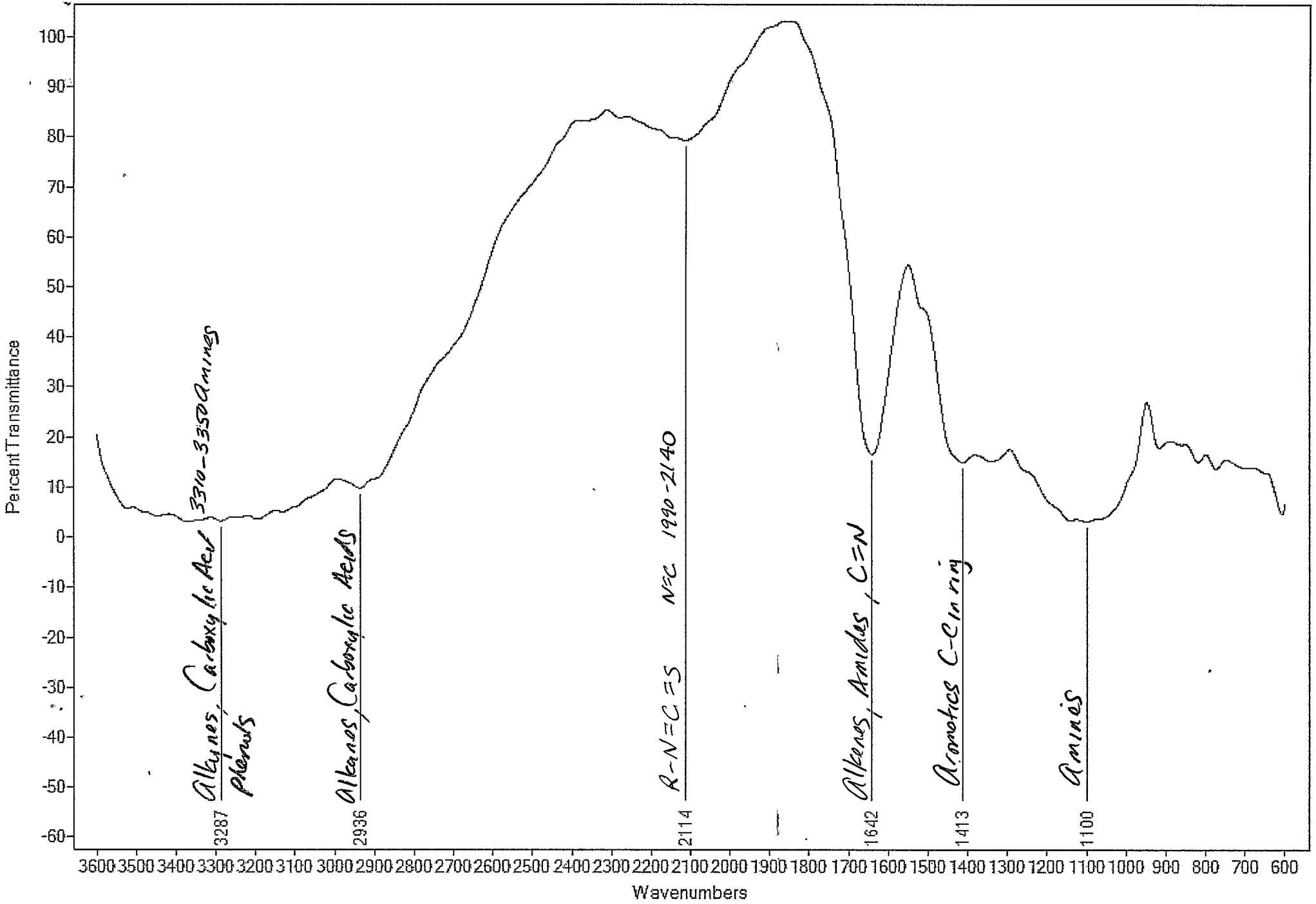
We cannot & do not assume the same for IR. The IR plots must be studied carefully.

However, with review, we can say that they are fundamentally the same.

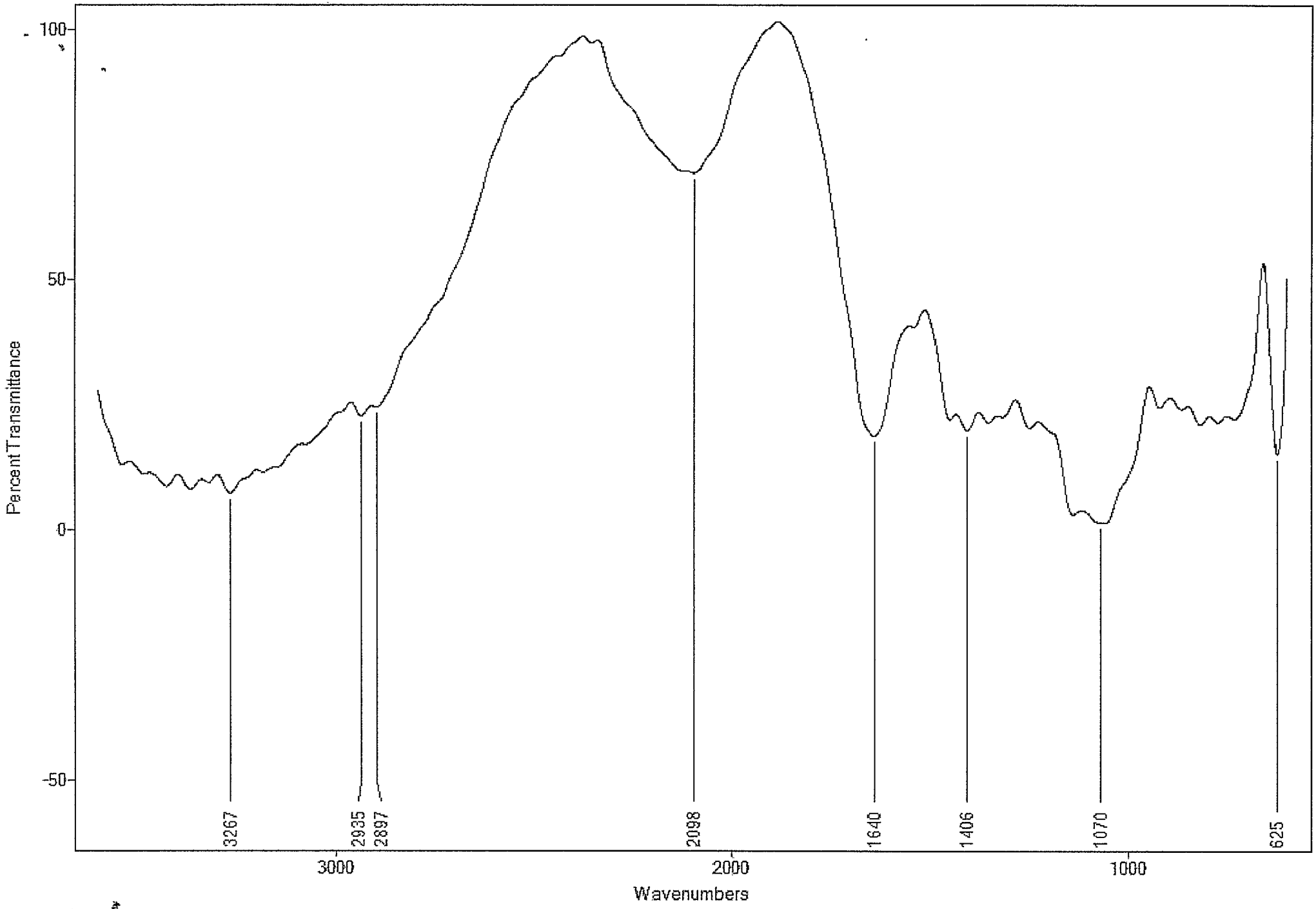
The CDB secreted protein, in its original form is fundamentally the same as the protein extracted and purified by liquid Chromatography, the source of the sample being a HEPA air filter operative for ~ 9 months.

On the following page we see further comparisons. They are fundamentally the same @ the functional group level.

CDB Concentrated Secreted Protein by Distillation Jun 14 2017 - 02.JPEG



CDB Secreted Protein Evaporated Jun 16 2017 - 05.JPEG



3-Way Protein Conformation Is Taking Place

The handwriting on the wall can be seen now. We already have confirmation existing between a long term air sample and the microorganism study with respect to protein production.

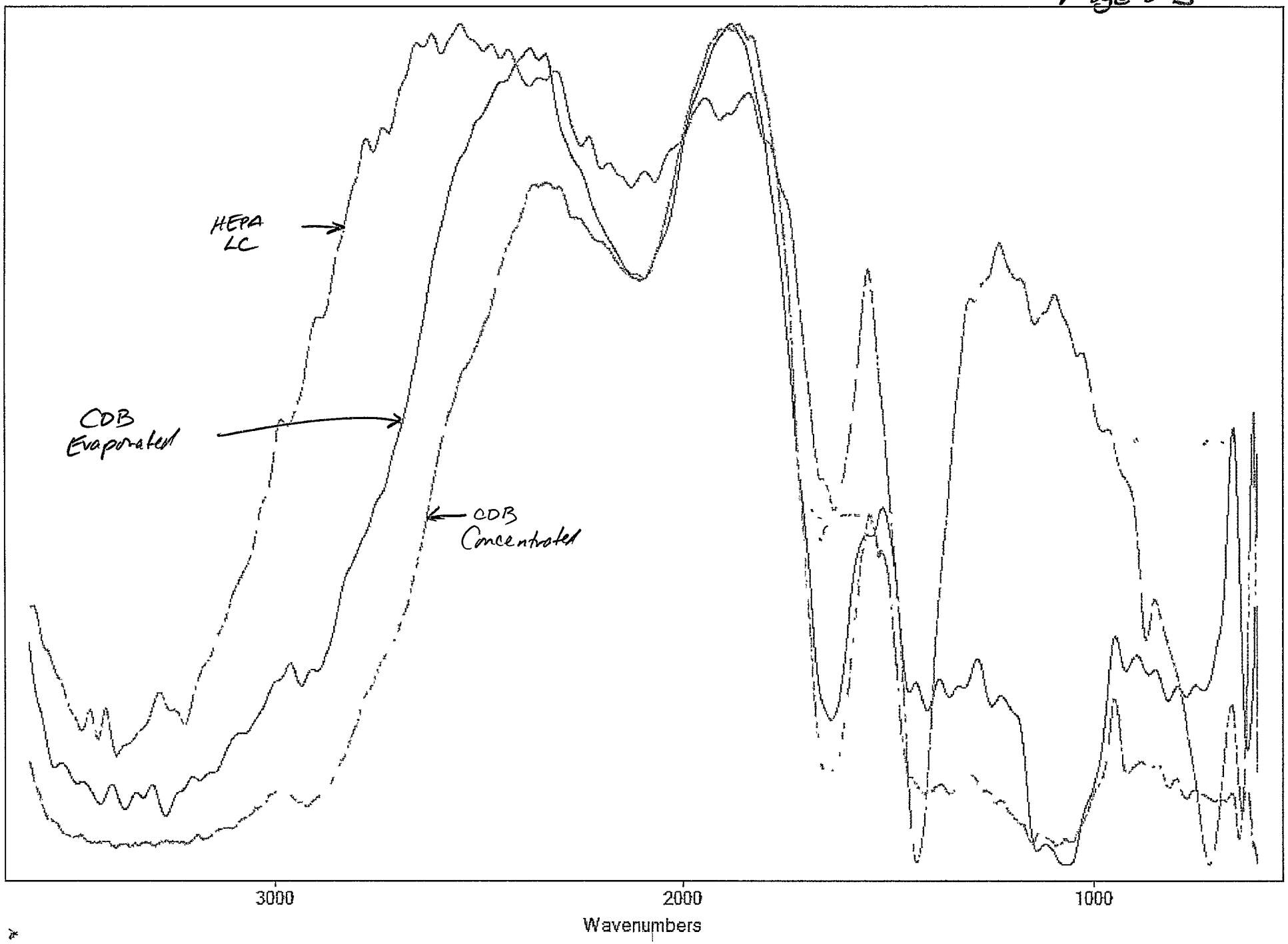
Now, with initial investigations into a rainwater concentrate sample, we already see that:

1. The UV spectra is identical to that of both the CDB secretion and the HEPA filter LC extraction.

This means that we have 3 way confirmation between source, air & water at the UV level.

2. We also have 3 way confirmation @ the Bradford test level. The rainwater concentrate clings to 623 nm and yields a positive test for protein.

3. We should all know what is next. Evaporation of the rainwater concentrate for IR analysis. The concentration of protein in/in the rainwater sample (concentrated by a factor of 14.55) actually seem relatively high. The rainwater appears generally clear but we have also seen color in samples that not clear that of the LC extracts.


HEPA LC
CDB Evaporated
CDB Concentrated
3000
2000
1000
Wavenumbers

3 way Protein Agreement: Page 24

The graph pretty much outlines the discovery

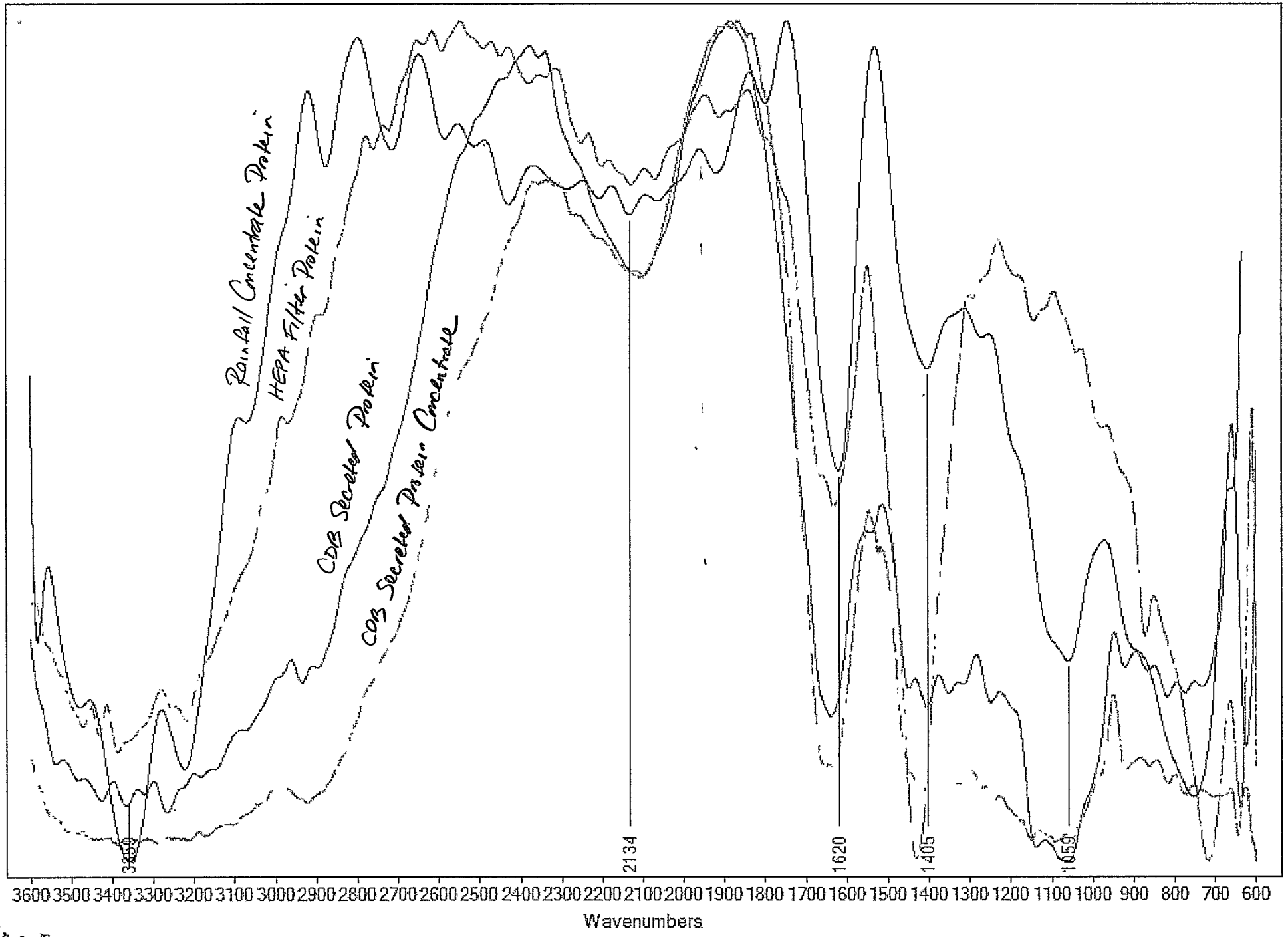
We have fundamental agreement in the functional group range (3600 – 1059 cm^{-1}) between three different proteins.

1. One from a microbial culture (CBB)
   re Protein secretion
2. The air (9 month indoor HEPA air filter)
3. The rain (rainwater concentrate)

Correspondence @ cm^{-1}:

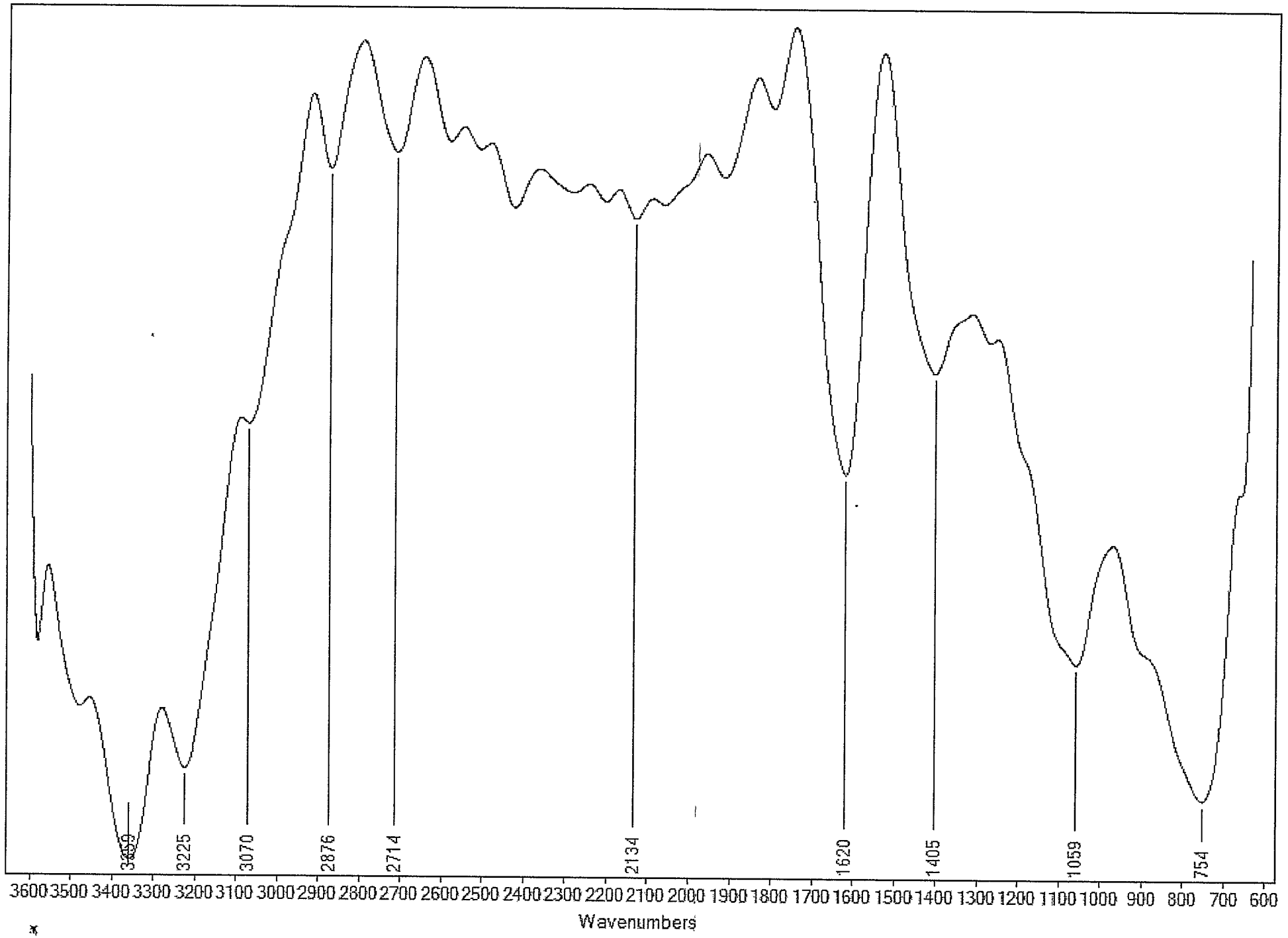
3350
2134
~1625
1405
1059

Rainfall Comparisons CDB HEPA Rainwater Jun 17 2017 - 01.JPEG



Rainfall Concentrate - Protein Jun 17 2017 - 01.JPEG

Page 25A



Orders

Jun 17 2017 Page 26

✓1. Tungsten bulbs coming - from Dubai ✓7. Silica gel
✓2. Digital thermometers are coming (4) ✓8. LC Column
✓3. 50-2ml amber bottles are coming ✓9. O_2 meter
✓4. Corks ✓5. KOH (2lb) ✓6. NaOH (2lb) USES

We have proven a fundamental level of protein equivalency between
1. CDB protein secretion
2. Air filter analysis - protein extraction
3. Rainwater concentrate - protein

Since the molecular weight may be quite low, technically we may be dealing w/ a polypeptide here vs a full protein. (10-100 amino acids)

In addition, the absorbance @ 235 certainly appears to be somewhat unusual and is ascribed to the presence of proline.

Proline is an unusual "amino acid" as it is technically not an amino acid.

It is a IMINO acid.

The only one of its kind in the group. 20 amino acids are used for biosynthesis.

All signs continually point to a genetically engineered bacteria.

Prolin undoubtedly offers many interesting avenues of research.

Evidence then for indicating the presence of these "amino" acids:

1. Tryptophan (aromatic)
2. Glutamic acid (Highly acid nature of secretion)
3. Prolene, an imine

We now have an interest in further analyses of the original COB protein, ie precipitated from water soluble form. The protein is different.

Lets regroup on the project list:

Projects

1st stage ✓ [redacted] book

2. Production of DNA

✓ 3. Additional analyses of original CDB protein

4. Molecular weight determination

Dry mt Specific Heat, Gravimetric — other possibilities? Titration, Dupont, Freezing Point

Looking very good 5. Elemental analysis approached again?

" 6. Carbon - Hydrogen ratio

" - combustion analysis?

7. Citizen samples

✓ 8. Molecular spectroscopy course

9. Start contacting DNA Labs

10. Monitor cultures

11. ICMP release

12. UV software purchase

13. GAMESS work continued

- NMR feller lead?

—

What strikes my fancy as to now look @ the original CDB extracted protein

MW of that protein? Needs to be dried and soluble in a solvent (pure)

✓ [redacted]

✓ 15. Does a dipeptide cause a Bradford shift?

16. Davis Courses

17. Specific heat - molecular mass rule of thumb

Dupont

Looking good 18. Combustion Analysis

Original CDB Protein Bradford Test.
Bradford Control values literature 633, 640, 636

$\bar{X} = 636$ nm

This come from Jan 09. 2017 & Jan 12. 2017

Now testing original CDB protein (precipitate from)
Used ~2 drops in 3 ml H_2O w/ 2 drops
1M HCl and then Bradford reagent.

We have a shift to 529 nm
Protein content is therefore verified. Shift is
not as strong as would be for more concentrated
sample but it is there nevertheless.
It also appeared to take a few minutes for
the color to develop fully.

Lets look @ how pH affect the solubility, esp
acid for the tumor lysis.

One of your tricks is to add 2 drops of conc.
HCl to cuvette w/ H_2O to neutralize coomassie
dye & stains for samples.

Lets try UV of our CDB protein.

1 drop of protein in 3 ml of H_2O does cloud
the solvent. HCl, conc. or dilute, does
not clear the solution.

strong alkalene, however, fully precipitate the protein and allows centrifugation to a clear solution. The precipitate changes color according to the level of alkalenity. At precipitation point, colors are aqua (est pH 7-5) at high alkalenity, the precipitate turns brown.

Notice that in neither case did the protein produce a clear solution in either acid or base, but that alkaline environment fully precipitate the protein.

Let's try acid again and see if the protein will separate by centrifugation.

Fascinating, it did precipitate out even in dilute HCl. This means that it is not dissolving in either case even though alkalenity is what caused it to originally precipitate out during the extraction process.

Now for Conc. HCl & Centrifuge

Yes, it is settling out w/ centrifuge even under conc. acidic conditions. This was not expected.

The means that during extraction already may have affected color & lack of reducibility more than solubility. That is an interesting question that could only be repeated by using the original extraction process; which is quite involved.

For now, this creates a motivation for us. The protein is not easily dissolved in either acid or base. Yet acid must be dissolving it partly because of the success of the Bradford Test.

Lets try this w/ UV

Setup:

4 ml H_2O	vs	4 ml H_2O
3 drops protein precipitate		
1 drop conc HCl		1 drop HCl conc as UV blank

Table Form:

	1	2	3	4
H_2O	✓	✓	✓	✓
HCl (1 drop conc)		✓		✓
Protein			✓	✓
> Centrifuge	Clear	Clear	Settles out	Settles out

Now centrifuge all four.

Now UV analysis..

First trial w/ H_2O blank.

Scan (1) vs HCl I did not expect to see anything but there is absorbance < 300nm. It is quite noticeable up to a peak of Abs = 0.64 @ 220nm.

The says that dilute HCl do not fluoresce under UV?

Lets repeat the sequence w/ HCl as the control & H_2O as the samples.

Indeed we did get a negative absorbance, a mirror image. The says that HCl does indeed absorb in UV and that the control solution is very important to establish properly.

Our reference obviously needs to be the H_2O + some 1 drop HCl solution.

A negative absorbance is not a problem as long as you understand how to interpret. It simply means that absorbance is less than that of the control.

React to H_2O + HCl control
+ in the future do not assume any solvent is neutral.

Now for centrifuged protein in conc. HCl against the control. We are after the solvent only, not the precipitate @ the bottom.

And we see that we have some very significant UV absorption here w/ peaks @
328 nm
223 nm

There is something significant is happening when you expose the protein to conc. HCl but there is no visible reaction. Notice also that there is no peak absorption @ 200nm / but that there is increasing absorption there.

So what exactly do we have here. How would the clear extract behave w/ Bradford test?

You would never be able to determine these types of results or situations without the availability of UV spectrometry. It is invaluable in circumstances like this.

What we do know is that at last the Bradford test invariably. There is supernatant is definitely something, but it is not protein.

Colby suggests napthol, aminobenzaldehyde as example candidates based upon UV peaks. Both have aromatics and OH on them.

It could be evaporated but it definitely is not the protein.
So the question is, how to dissolve the protein & prepare it for IR?

pH is way too low for ATR: It is @ 2.5
so it must be raised to mid range.

Today we learn some very important things from protein comparisons.

It is true that proteins will share a great deal of common ground upon the functional group region. However even upon this region there can or will be important differences.

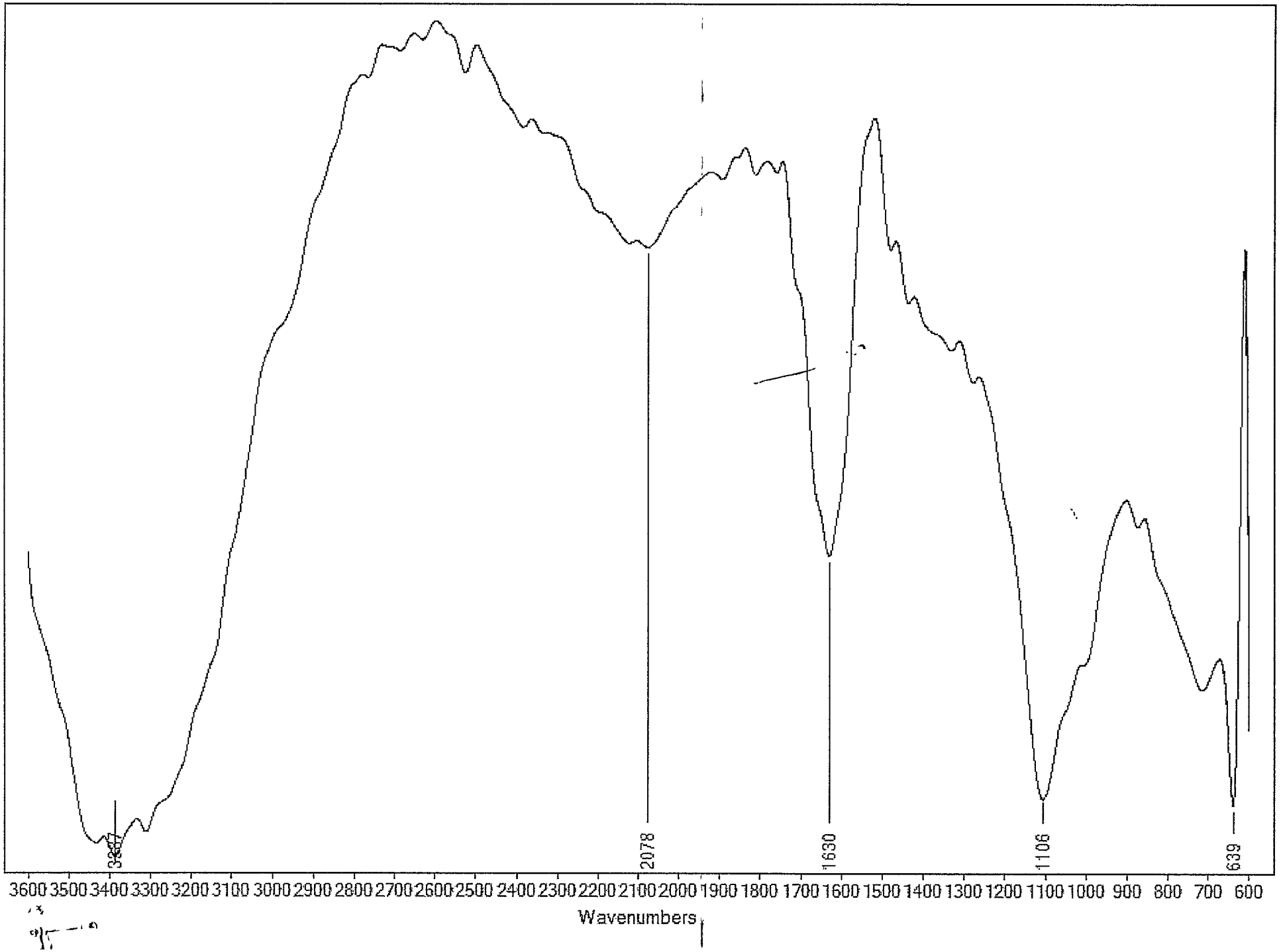
You have just cause to have interest and some concern on the dominance of the proline group (~2100). You can see all of your proteins that do represent a very strong absorbance region.

However, if you compare:

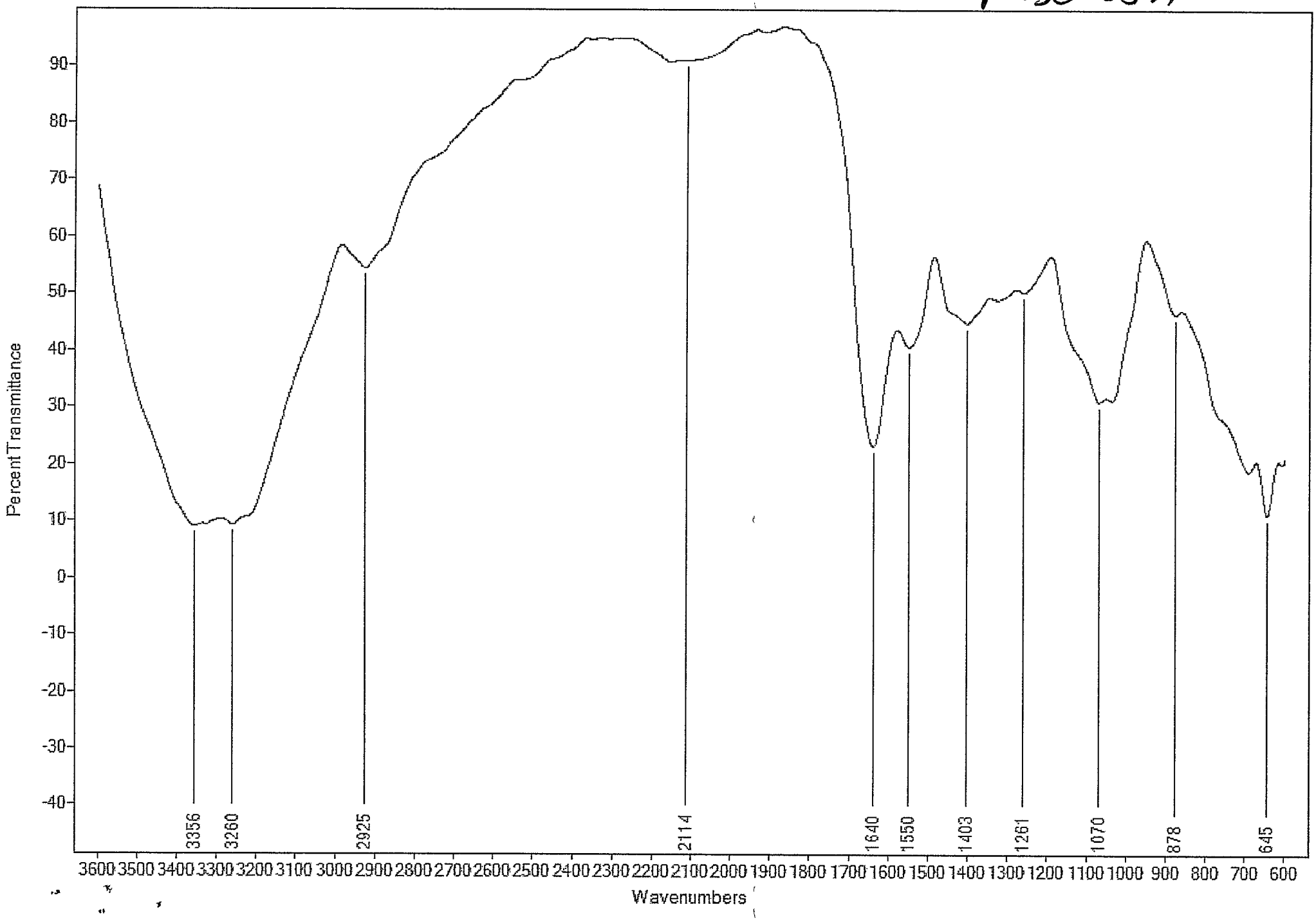
1. Blood & saliva
   You will find there is a very big difference. Blood & saliva is known to have an abundance of Proline rich proteins (PRP) but blood is not
2. CDB proteins & powdered milk
   You can see the major absorbance on the CDB protein set vs the comparison powdered milk trial

They are not the same. Proline dominance & strength with your CDB / environmental proteins is a legitimate concern.
There is a question about how the mylk affect the blood.

CDB Precipitated Protein Jun 17 2017-01.JPEG

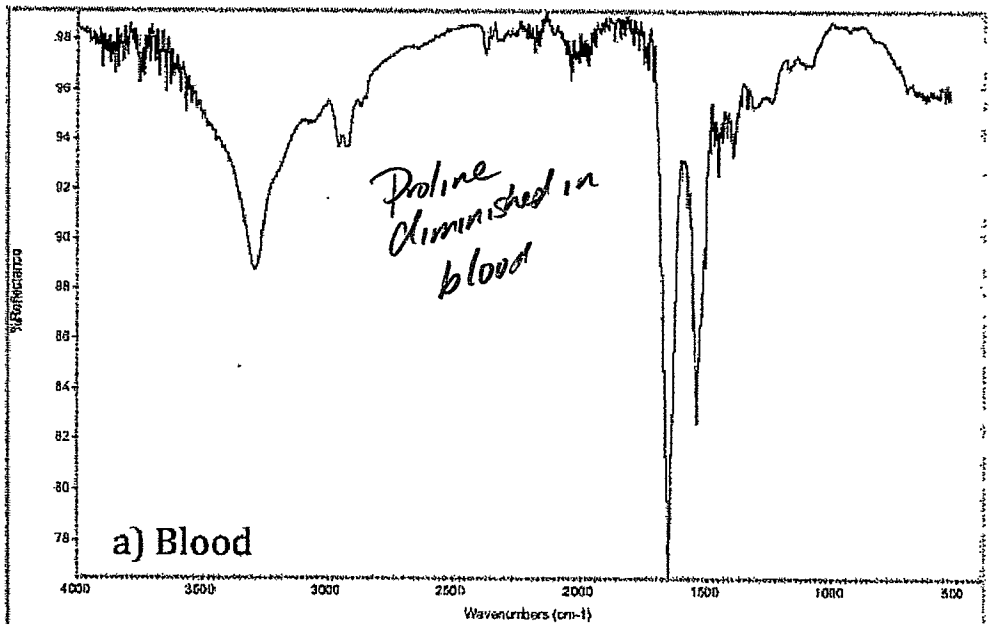


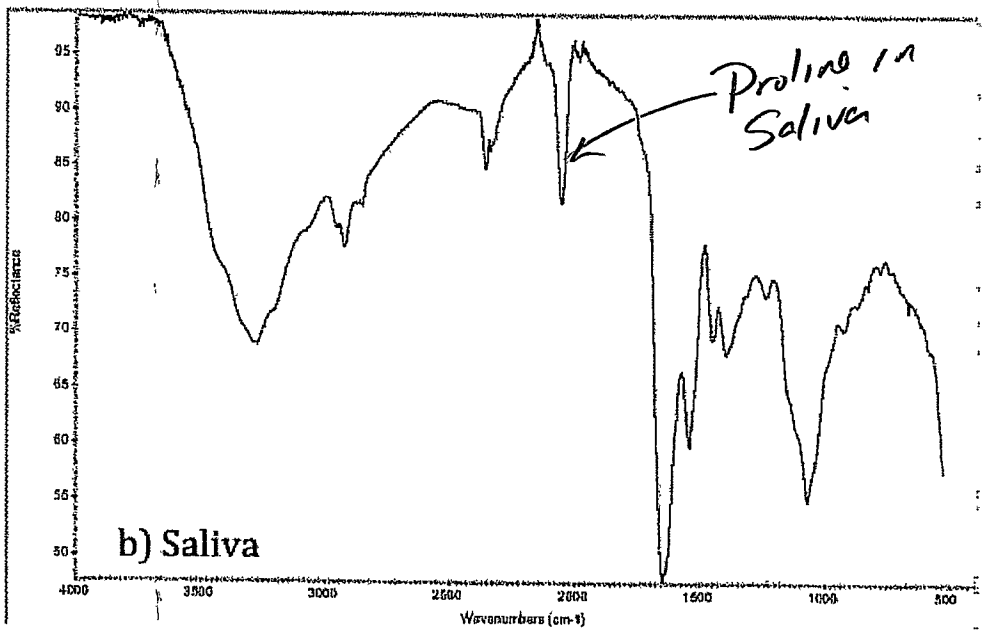
Powdered Milk Protein Jun 17 2017 - 02.JPEG



Proline Comparison between blood & saliva

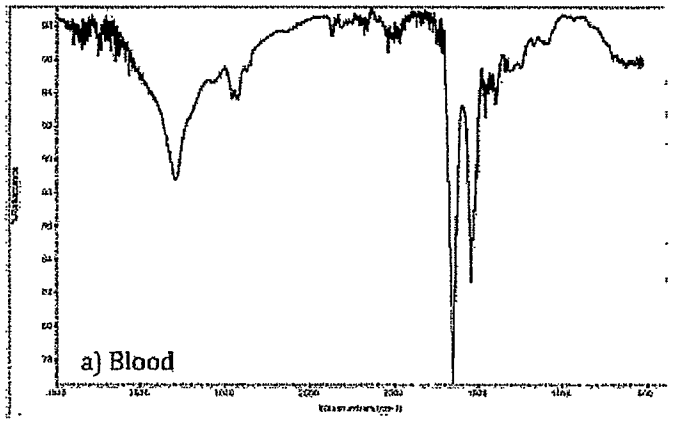
Untitled - 1

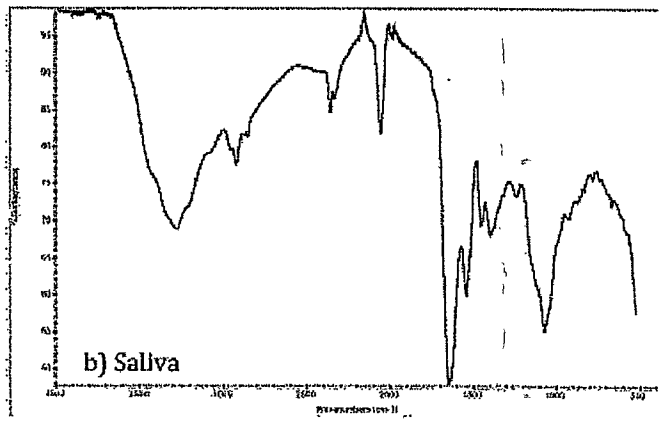


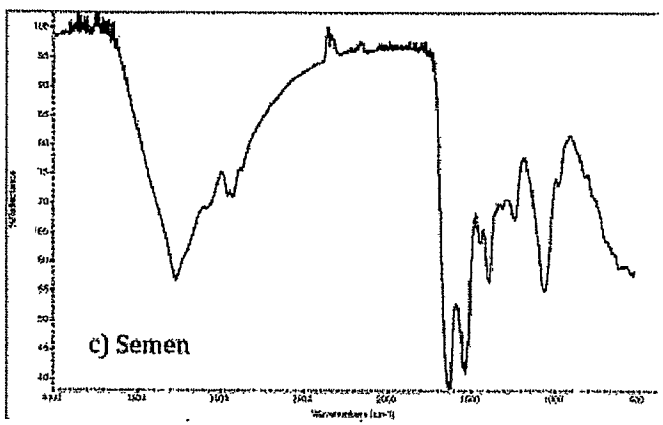


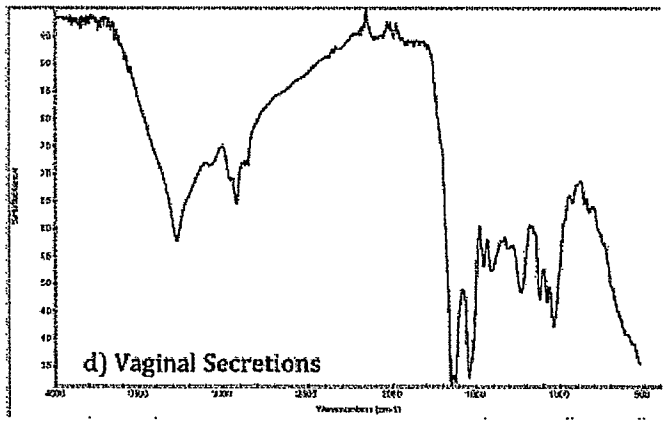
From Forensic FTIR Book

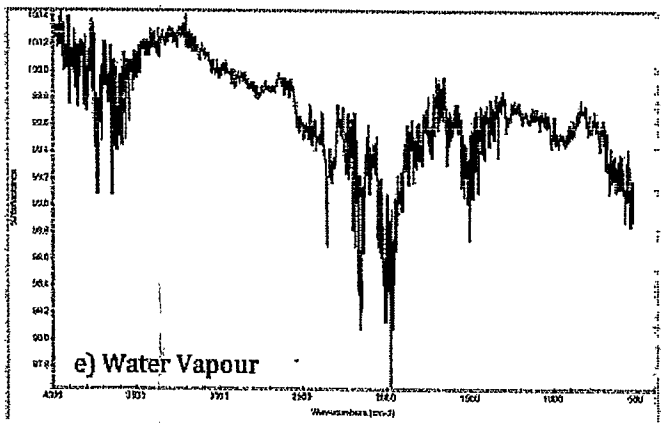
Untitled - 2











You also learned today that ATR has its advantages & disadvantages. Sometimes it is complementary to the spectrum and other times it is not.

A major advantage is that you do not need to thoroughly dry the sample. Removing most of the water seemed to suffice w/ the use of the glass slide or a powdered milk concentrate sample.

There was no real advantage in using either a
1. ATR glass slide blank Control
or
2. an ATR glass slide w/ water blank Control

Your best results were obtained using an ATR air blank Control, even when the sample was partially water (powdered milk concentrate) with a glass slide.

averaging both KCl & ATR spectrum did produce the best result.

We now have much if not most of the protein segment in our hand. You are well equipped to proceed onto the paper, if you so choose, entitled "The Proteins Amongst Us"

Does a dipeptide show the Bradford shift?
Assume Control λ_{max} = 636 nm.

We definitely do have a shift to 631 nm.

Thus means a dipeptide will meet the Bradford test.
Aspartame used as the final sample.

Jun 18 2017 Balance in this order:
Combustion Analysis: Carbons
Combustion reaction Hydrogens
Oxygen

$C_xH_y + O_2 \rightarrow H_2O + CO_2$

Eg

Methane $CH_4 + 2O_2 \rightarrow 2H_2O + CO_2$
③ ② ①

Pentane $C_5H_{12} + 8O_2 \rightarrow 6H_2O + 5CO_2$

~~2.62~~
~~2.27~~ gms sugar
Empty tube weighs ~~10.30~~ gms 22.57
tube w/ blank cork weighs ~~17.13~~ 23.39
Wgt of cork = ~~0.83~~ gms 0.82
Wgt w/ cork and sugar = ~~20.61~~ gms 24.68

Wgt of sugar = ~~20.61~~ 24.68
~~- 17.13~~ - 23.39
~~= 3.48 gms~~ 1.29 gms

Ok, way (weigh!) too much sugar, and way too hot. Ease off.

Be very careful w/ your custom glass tubes. Heat gradually & carefully. Sugar is a very difficult compound to burn completely. Your custom tube does not need to be strength tested.

Final tube w/ ash weighs 22.71 gms
Therefore wgt of ash is 22.71
-22.57
0.14 gms

Therefore 1.29
-0.14
= 1.15 gms is total organic carbon

and 0.14 gms is supposedly inorganic (10.8%)
which is not really true.

The empty tube now weighs 22.57 gms again
so everything is clean and fine again.

Now the will tell you how much is organic &
how much is inorganic. In this case the
residual sugar ash of 0.14 gms was subjected to
further direct heating w/ the torch and all of
it combusted. Therefore both of the
sugar is all organic, which is true.

What we do not know is the ratio of carbon
to hydrogen.

You must collect the CO_2 & H_2O to get this
information. Magnesium sulfate is a great drying agent! 1st tube

NaOH absorbs CO_2 (2nd tube)
$Ca(OH)_2$ also absorbs CO_2 and forms $CaCO_3$

Ammonium sulfate should absorb water? Hygroscopic above 81% humidity
@ room temp

The special cork weighs 0.80 gms.

The 1st tube (for H_2O) w/ $MgSO_4$.
weighs 16.25 gms (no corks) 19.03

H_2O $MgSO_4$ tube (no corks) loaded weighs ~~18.96~~ 18.98 gms

This means $\Delta MgSO_4 = 18.96 - 16.25 = 2.71$ gms dry 19.03 2.78
added H_2SO_4.

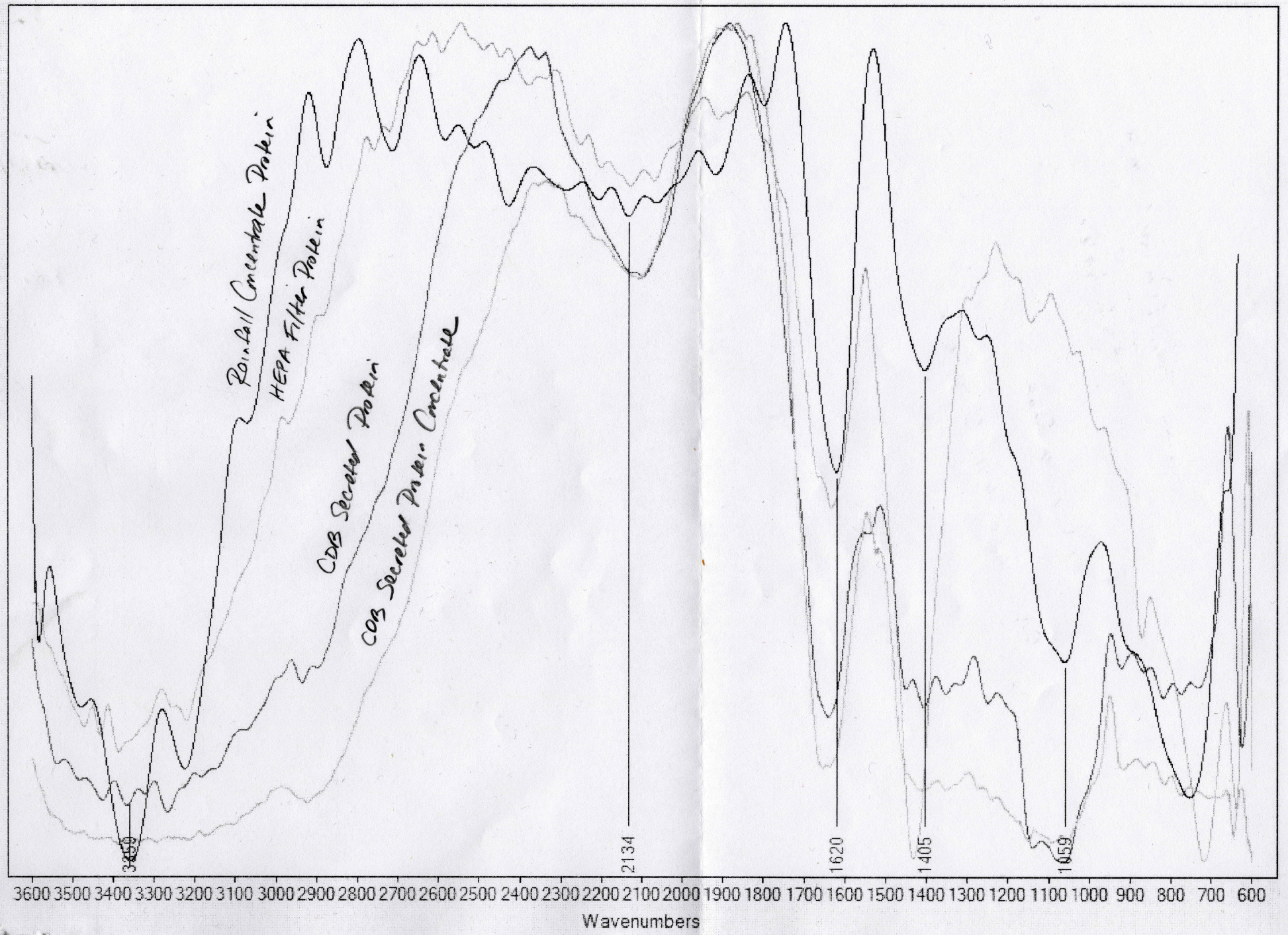
CO_2 2nd Tube $Ca(OH_2)$ unloaded, no corks
weighs 16.82 gms

The $CaOH_2$ loaded tube (no corks) weighs
18.68 gms.
Therefore $\Delta Ca(OH)_2 = 18.68 - 16.82 = 1.86$ gms dry

The sugar tube loaded weighs ~~26~~ 23.67 gms
Empty sample tube 22.57
Δ 1.10 gms

Post.
Water tube 19.30
CO_2 tube 18.74
Sample tube 22.71

Rainfall Comparisons CDB HEPA Rainwater Jun 17 2017 - 01.JPEG



Sample: 23.67 pre combustion
-22.71 post combustion
.96 was burned off

Original mass was 1.105 ms
- .96 burned off
.14 remains as ash (incomplete combustion)

This means that we have (.96/1.10) = 87.3% combustion
(.14/1.10) = 12.7% incomplete or inorganic.

Now our water tube pre: 19.03 pre combustion
19.30 post combustion
.27 gms H_2O collected

CO_2 tube: pre 18.68 pre combustion
18.74 post combustion
= 0.06 gms CO_2 collected

all the carbon ended up as CO_2 & all the hydrogen ended up as H_2O
(.2729)

Carbon: .06 gm (12.011/44.0098) = .0164 gms C
Hydrogen: .27 gms (2.0158/18.0152) = .0302 gms H
.0119

Moles Carbon: .0164 gms / (12.011 gms/mole) = .00136 moles
moles Hydrogen .0302 gms / (1.0079 gms/mol) = .0300 moles
Our ratio here is CH_{22} which obviously does not work. Susan syringe kicked over into collection tube and distorted water.
We should be producing substantially more CO_2 than H_2O.

Do we do have some problems of course
Sucrose is $C_{12}H_{22}O_{11}$

So the CH ratio should have been almost 1 to 2

Sugar has a lot of oxygen attached to it

~~It would~~ so then we are complicated than we want right now.

Δ			
1.37	Sample tube 24.43 loaded w/sugar	no corks	23.06
.06	(1) $MgSO_4$ tube: 18.43 loaded	"	18.49
.01	(2) $CaCl_2$ tube: 17.95 loaded	"	18.02

Sample 24.43 − 22.57 = 1.86 after
.49 gms remains in tube ~~after~~ it melted

C: .06(.2729) = .0164 gms
H: .01(.1119) = .0018

C mole = .0164 / 12.011 gms/mol = .001365 mole
H mole = .0018 / 1.0079 gms/mol = .00174 mole

Ratio = 5.67
Ratio should be almost 2 to 2. (actually 1.83 to 1)
so then we @ least were better
off by a factor of ~3 instead of 22!

Ok, you have done better this time.
but the $Ca(OH)_2$ still needs about more,
about 3 times more than it does
but if you can start to establish repeatability
that would be a good thing done.

Assume
C: $\Delta = .0018$ gms $\Rightarrow .0018(.2729) = .0049$ gms
H $\Delta = .07$ $\Rightarrow .07(.1119) = .0078$ gms

C: mole: $.0049 / 12.011 = .0041$ moles
H: mole $.0078 / 1.0079 = .0077$ moles

Ratio = 1.88 to 1 Actual is 1.83 to 1
So yes, close as what you need.

$C_1H_{1.88} \Rightarrow C_{12}H_{22.56}$ This is where we need to get to.
$Ca(OH)_2$ tube is off by a factor of 1/3. But better now.

It may have helped to dry the $Ca(OH)_2$
do the run longer next time.

Empty sample tube is now 22.71 gms
17.30 $Ca(OH)_2$ pre ~~17.30 post~~ 17.32 ~~.00~~ .01
17.33 $MgSO_4$ pre 17.36 post .03
24.90 Sample
.078 Need this not 0.0 .00143 .063(.2729)/12.011
C: $.01(.2729) = .00273$ gms $\Rightarrow .000227$ moles Ratio = =
H: $.03(.1119) = .003357$ gms $\Rightarrow .00333$ moles
No good $Ca(OH)_2$ needs to have D of about 6.3 to 1.
2.6

			Δ
Empty Sample Tube	16.00		
Sample Tube Loaded	16.05	16.93	
$MgSO_4$	19.51	Loaded 19.63	.06
$Ca(OH)_2$	18.49	Loaded 18.52	.03

Assume $Ca(OH)_2$ needs factor of 3 = .09
Actual Factor needed ~~is~~ ~5.2
You do not know that w/out using as a control

			Δ 6Δ
Sample Tube Empty	22.18		
Loaded	23.60		
$CaOH_2$ loaded	18.52	18.55	.03 .18
$MgSO_4$ loaded	19.63	19.70	.07

Ratio = ~~2.33~~ 2.43 Factor of 6.3 required
This is not too bad. Ratio needs to be about +2%
Best so far

$C: [(\overset{6.3}{1})(.03)(.2729)] / 12.011 = \overset{.0043}{.0041}$ moles

$H: [(.07)(.1119)] / 1.0079 = .0078$ moles

Ratio = $\overset{1.81}{1.79}$ Should be 1.83 to 1 $\cong C_{17}H_{22}$

It is now clear that the $Ca(OH)_2$ needs to be allowing 6.3 times as much CO_2 as it does

Twice now ya have an underweight of $Ca(OH)_2$ massively a factor of ~6.

No idea why — is there something else that will absorb CO_2 that ya have?

$C_xH_y + O_2 \rightarrow H_2O + CO_2$

The balances of the equation varies depending upon what C_xH_y is.

Try it w/ KOH or NaOH to remove CO_2

	PRE	POST	Δ	Total accounted for.
Sample Tube 22.24 Empty				.06 + .19
Ris	19.68	19.74	.06	+ .31 = .56
KOH	20.01	20.20	.19	
Sample Tube Loaded 24.58				2.34 Sample
ratio = 3.2				.96 Total
				Δ = 1.76 missing?

C: $0.19(.2729)/12.011 = .00432$ moles

H: $.06(.1119)/1.0079 = .00666$

Ratio = 1.54 Ok getting more better!!!

For the first time ya need more water absorbed!!!

Dry out to $MgSO_4$ next.

Have $C_{12}H_{18}$ Not bad! Ya are getting better now.

Sample Tube Post = 22.55 Δ left = 0.31 gm

KOH ya superior

Jun 19 2017

I have completed the Molecular Spectroscopy course today from Univ. of Manchester. Good work. I am much better prepared w/ NMR now, in addition to UV & IR.

I am starting to make some progress w/ the Carbon-hydrogen ratio (empirical formula) problem. It is difficult w/ DIY simple equipment but the methods are improving.

It is necessary to heat slowly and evenly — the lines are not capable of handling heat as well as I desired. I will try a metal sample tube @ some point.

KOH (also NaOH) has worked much much better than the $Ca(OH)_2$ approach for CO_2 trapping. You actually now need to improve H_2O collection which you will try by drying the $MgSO_4$ immediately prior to use.

You have simplified the calculations considerably now.

you are investigating combustion analyzers.
Use the fuel type selection only for
advice on recommended readings or does
it affect the actual data obtained.

Can you use the data of a combustion analyzer
to assist in elemental analysis?

I am also headed toward investigating the GC TOC
for similar purpose and end

$$C_xH_y + O_2 \xrightarrow{\text{heat}} H_2O + CO_2$$

Every situation is different for balance purposes.

General idea: If you know resulting masses of
collected H_2O & CO_2 you can determine the
no. of moles from the mass fractions of H & C.
You have direct measurements in this case.

Combustion analyzer will measure 1 or two gases
such as CO_2. But how would you ever get hydrogen
out of the mix; it does not measure H_2O.

But actually GC does measure H_2O w/ a sloppy
and trailing peak. You will never have complete
combustion but these two gases (H_2O vapor & CO_2)
should still be measurable

Another issue is that you are only collecting a small portion of the original mass so most of the sample is passing through uncollected.

A problem w/ GC TCD is that the nitrogen peak is going to overlap the ~~nitrogen~~ O_2 peak so I do not think that you can separate it.

You also learned about DuPont-Petit Dupont-Petit specific heat – molecular mass relationship. (rule of thumb)

Jan 20 2017 CH Ratio - MW Estimate (Cont)

	Pre	Post	Δ
Empty sample tube:	22.30 gms		Residual 0.23 gms
Sample tube loaded:	23.61 - (Δ=1.31)	22.53	1.08 gms
KOH loaded	20.36	20.52	.16
$MgSO_4$ loaded	22.22	22.30	.08

C: $\emptyset.16(.2729)/12.011 = 3.64E-3$ moles

H: $\emptyset.00(.1119)/1.0079 = 8.00E-3$ moles

Ratio = 2.26 In this case too much H_2O, not enough CO_2 but not bad.

Your most recent value as Ratio of 1.54
2.26
$X = 1.90$

$C_1 H_{1.90}$

$C_{12} H_{23}$ You are definitely getting there - Very good work.

You also can estimate molecular weight by two methods:

Freezing pt. depression & partially specific heat

You already have a molecular weight estimate of: We found a MW estimate of 359 gms/mole (Lactose is 342)

So

$C_x H_{1.9x} \cong 359$ gm

$X(12) + 1.9(1)x \cong 359$

$X(12 + 1.9) = 359 \Rightarrow X = 26$

This would lead to $C_{26} H_{49}$

But what we actually have is

$C_x H_{1.9x} O_z = 359$

$X(12) + 1.9(1) + z = 359$

~~But the rule of 13 can be used to estimate~~
But then we would have

$X(12) + 1.9(1)X + z(16) = 359$

$13.9X + z(16) = 359$

We observed significant moisture is still coming out of the $MgSO_4$ so there was a good idea.

KOH was heated up so it did need to be refreshed. This means you will need to purchase chemicals which does require a commercial address.

KOH should also be dried. I found a source on ebay. Also in order of 2 lb NaOH. I also have found nitric acid.

Wait until you get your corks to fit metal combustion chamber.

A

Copper tube empty	102.30	103.33 loaded	
MgO_4 loaded	22.12	22.25	need abt .01 Ø.13
KOH ~~21.70~~	21.69	21.87	Ø.18 or abt Ø.34

Better recovery of material Ratio = 1.38

C: (Ø.18) (.2729) / 12.011 = 4.09E-3 moles

H: (Ø.13) (.2119) / 1.0079 = 1.44E-2 moles

Ratio = 3.52 which is now too high.

Not enough Too much water or not enough carbon

C_{12} H_{42}

I would say your water collection looks too high.
That means we will not dry it so much.
Could be it has absorbed moisture from the air.

In general, good work though.
Now using a metal tube
Need to keep the ends cool

The question now is whether the $MgSO_4$ was too dry and now absorbed moisture from the air in addition to that of the sample.
Since CO_2 quotient looks like improved maybe $MgSO_4$ @ normal room conditions is OK?

Page 52

	Pre	Post	Δ
Empty Sample	101.97		36% used.
Loaded Sample	103.97	103.25	~~36% used~~
~~Empty~~ Pre KOH	21.88	22.10	Ø.22
Pre $MgSO_4$	22.24	22.36	Ø.12

Ratio = 1.83

C: (Ø.22(.2729))/12.011 = .5E-3 M
H: (0.12)(.1119)/1.0079 = 1.33E-2

Ratio = 2.67 ~~Kotta, we have it.~~ ∴ Still too high.
This ratio should be approx 1.86
$C_1H_{2.67}$ ⇒ $C_{12}H_{32}$ vs $C_{12}H_{22}$
So you are still absorbing too much moisture. It should be about Ø.085 or about 50% too much.
I wonder why this is?

The tube handled the heat well. The cooling strips on the end worked very well. There was no smoke however, which was discreetly. CO_2 plug?

Try again w/ fresh materials $MgSO_4$ room conditioned.

			Δ
Sample empty	102.04		
Sample Loaded	104.08	103.21	
KOH	20.93	21.06	Ø.13
$MgSO_4$	22.39	22.52	Ø.13

Not good. You have an imbalance. KOH looks good but Δ mass does not reflect much change.
Notice MgOH is almost identical each time Ø.13, Ø.12, Ø.13
The part we plate well. but KOH: Ø.18, Ø.22, Ø.13
always too low.

Ratio of original mixtures should be 1.03

MgSO4 mean = 0.127
KOH mean = .177

Notice it is off by a factor of 2

KOH should be 1.03(.127) = ~~.232~~ ~~0.33~~ 0.32

.34

So K is off by a factor of $\frac{.232}{.177} = 1.31$

((.2729)/12.011 = 2.27E-2

$\frac{2.27E-2}{\frac{x(.1119)}{1.0079}} = 1.03$ ∴ $\frac{2.27E-2}{\frac{1}{x}(.1119)}\left(\frac{1.0079}{}\right) = 1.03$

$x = \frac{2.27E-2(1.0079)}{(1)(.1119)(1.03)} = .112$

.34 ~~0.32~~

C: ~~0.33~~(.2729)/12.011 = ~~7.56E-3~~ 7.73E-3
H: .127(.1119)/1.0079 = 1.41E-2

Ratio = ~~1.86~~ 1.82
12(1.82) = 22 OK

The ~~is~~ extracting KOH is off by a factor of almost exactly 2

Factor of 2 is required! in KOH

Write out the reaction between CO_2 & KOH

$$CO_2(g) + 2KOH(aq) \rightarrow K_2CO_3(aq) + H_2O(l)$$

Notice this is not balanced

1 C both sides now it is balanced.
4 O vs 4 O
2 K vs 2 K
2 H vs H_2

And guess what? 2 moles of KOH are used for every mole of CO_2

You apparently must balance

Assume an measure KOH $\Delta = .18$ $2(.18) = .36$
$MgSO_4 = \Delta = 0.13$ .13

C: $0.36(.2729)/12.011 = 8.18E-3$ moles
H: $0.13(.1119)/1.0079 = 1.44E-2$

Ratio = 1.76 Ok, now you are on the right track
$C_{12}H_{21}$

So the problem was: 1 mol. of produced K_2CO_3 uses 2 moles of KOH, so you need to double = your measurement change

I have C/H ratio coming in now!

It does seem as though repeat trials are required to reduce the error, especially in KOH - CO_2 use.

H_2O consumption seems to be reliable.

Therefore our average value are

$\Delta MgSO_4 = 0.1279$ gms

$\Delta KOH = 0.1779$ gms $\Rightarrow \times 2 = .354$

C: $.354(.2729)/12.011 = 8.04E-3$

H: $.127(.1119)/1.0079 = 1.41E-2$

Ratio = 1.75 a little low, it should be 1.83

leads to an estimate of

$C_1H_{1.75}$

$C_2H_{3.5}$

$C_3H_{5.25}$

C_4H_7 this is our estimate of the empirical formula.

The actual answer for sucrose is $C_{12}H_{22}O_{11}$

so we would end up with

$C_{12}H_{21}$ vs. $C_{12}H_{22}$ not bad!

We also have a molecular wt estimate of 359 (actual is 342)

So our notation is
$[(C_4H_7)O_{\square 2}]_n = 359$ gms

$C_{12}H_{21}O_n = 359$

$= 12(16) + 21(1) = 213$ $\quad 359 - 213 = 146$

$146/16 = 9$

So we would be led to believe we have

$C_{12}H_{21}O_9$ for our compound. What does this come look like? $\quad$ Actual is $C_{12}H_{22}O_{11}$

This is not bad

You can allow slack on atoms & MW on SOBS and do some clearly.

C_{11} to C_{13} $\quad$ MW 320 to 360!!
H_{18} to H_{24}
O_8 to O_{10} $\quad$ 9 compounds show up.

Jun 27 2017

Seeley Lake MT:

Vicki Dolar = Analytical Chemistry Course = Coursera

Revisiting the class, Vicki has very clearly explained the intimate of liquid Chromatography the discussion will also apply very well to GC

3 Parts:

1. Column (or Stationary Phase)
2. The analyte (what you are trying to separate & identify)
3. The mobile phase, ie, the solvent

in GC, the mobile phase is a gas, and an inert one @ that.

The interactions of 1, 2 & 3 will define your results and success in LC, and the understanding of these interactions

Columns can be polar or non polar, and also acidic or basic

Alumina is apparently moderately polar & acidic. Silica gel is highly polar and basic.

Mobile phases are either strong or weak. Strong mobile phases are of the same character as the column. Since they are so busy interacting w/ the column, they "block" the analyte from interacting w/ the column and the analyte then passes through

the column very quickly. You do not generally want this behavior.

If the mobile phase differs more markedly from the column (ie unreal) and primarily in polarity) then the mobile phase will not interact with the column as much and the analyte will have the opportunity to do so (depending upon its polarity also of course).

So to interpret some results that we already have w/ the HEPA pellet extract (dissolved in wet methanol). We used both water as the mobile phase, a strong acid and a strong base. Water produced a very quick eluent and base produced a secondary & delayed eluent.

Therefore:
Alumina (aluminum oxide) is apparently moderately polar. A mobile phase of water as the solvent is also polar, probably even more polar than the column stationary. The analyte therefore did not have much of an opportunity to interact w/ the column and so it eluted almost immediately. We know therefore, that the analyte is highly polar in nature.

Now we also noticed that a colored material was retained on the alumina column. Alumina retains acidic substances (silica gel retains basic compounds) + as alumina is acidic also.

Acids react w/ bases. Thus as when you added a strong base you did an ionic reaction and material was eluted from the column. You know, therefore that the analyte is of an acidic character, & added we now know it is a highly acidic protein.

So now you can interpret the LC results more clearly and you can act in a more predictive state w/ LC results w/ this knowledge.

GC is going to have a similar rationale but it is even simple to understand since the mobile phase (gas) is inert to the stationary phase in that case.

A polar LC column is called "a normal phase column"

A non polar LC column is called a "reverse phase column".

Clearly it would be of great benefit, in terms of flexibility to have a non polar LC column to work with.

They are made by binding C8 or C18 chains bound to silica.

"It is slightly more difficult and expensive to obtain a column where the stationary phase is non polar"; as all solid adsorbents are polar by nature.

What is done is to coat silanized silical gel w/ a non polar liquid".
(Silicone & various hydrocarbons)

Our paper here came from the Univ of Idaho
Titled Lecture 36 Combustion Reactions

Jun 28 2017 Page 61

I believe that I may have a second method to determine the C-H ratio, this time w/ GC.

Combustion reactions are quite interesting and well defined.

The essence will be that:
"For every mole of oxygen involved in a Combustion reaction, THERE ARE 79/21 = 3.76 moles of nitrogen."

Here we can measure the combined N_2-O_2 peak in GC-TCD and we can measure CO_2. I believe we should be able to get the CH ratio.

Complete Combustion is

$$N_2 + O_2 + C_xH_y = H_2O + CO_2 + N_2$$

Heat

$N_2 + O_2$ — We can measure this sum but only after the reaction.

But we can also say for every mole of nitrogen involved in a combustion reaction, there are 21/79 = 0.266 moles of oxygen.

We may or may not be able to measure H_2O.

$C_xH_y + n_0(O_2 + 3.76N_2) \rightarrow n_1(CO_2) + n_2(H_2O) + n_4(N_2)$

C: $X = n_1$ or $n_1 = X$] This is what is most critical to know

H: $y = 2 \cdot n_2$

O: $2n_0 = 2n_1 + n_2 + 2n_3$. n_3 corresponds to coefficient of SO_2 produced.

this means that $n_0 = \frac{(2n_1 + n_2)}{2}$ with no sulfur involved.

I have always wondered why chemical balancing of chemical equations was not handled in this fashion more often.

Now what can we measure?

1. An area for CO_2
2. Possibly an area for H_2O (You could calculate it since combustion)
3. The area of Nitrogen & oxygen together, but not oxygen or nitrogen alone (Prior to combustion!)

I think we also know that $2n_0(3.76) = 2n_4$

or

$3.76n_0 = n_4$ (from prior to combustion)

We also know n_0 (prior to combustion)

that means we should know n_4 after combustion

$n_4 = 3.76n_0$

If it is a pure hydrocarbon, we know that $n_2 = 2n_0 - 2n_1$

Since we know n_0 (prior to combustion) and we know n_1 by msmt, we should be able to determine n_2!

The process can be started w/ oxygen
There are some problems however in maintaining a
Constant volume of gas into the instrument
But in theory

1. Measure the size of the O-N peak prior to Combustion

2. Measure the size of the CO_2 peak after

3. Measure the H_2O peak after (if possible)

4. Attempt two methods
   1. Direct measurement of area ratio CO_2 to H_2O
      (I love to wonder about the complications of failing)
   2. Area of H_2O = ? [2 · Area of (N_2 + O_2) prior to Combustion] − Area of CO_2 (post Combustion)

Is this true?

You may have up to 3 methods of determining the CH ratio FOR A PURE HYDROCARBON.

Development of a Gas Chromatography Method of Combustion Analysis

However, note that combust in w/ excess oxygen (which we have) yields products of CO_2, H_2O, N_2 & O_2

Our next most involved situation is:

$$C_x H_y S_a O_z N_b + n_0(O_2 + 3.76N_2) \Rightarrow n_1 CO_2 + n_2 H_2O + n_3 SO_2 + n_4 N_2$$

Relationships already known are:

C:	$x = n_1$	5 equations
H:	$y = 2n_2$	5 unknowns
S:	$a = n_3$	
O:	$z + 2n_0 = 2n_1 + n_2 + 2n_3$	
N.	$b + 2(3.76)\cdot n_0 = 2n_4$	

We can measure n_1 so this leads to 4 eq., 4 unknowns.

If there is no S, we know $a = n_3 = 0$.

This leaves us with:

C:	Determinable	
H:	$y = 2n_2$	n_2
O:	$z + 2n_0 = 2n_1 + n_2$ but n_1 is known, so	~~n_0, n_2~~
O:	$z + 2n_0 =$ Constant $+ n_2$	n_0, n_2
N.	$b + 2(3.76)n_0 = 2n_4$	n_4

or now, 3 equations w/ 3 unknowns:

n_0, n_2 & n_4

We should know n_0 prior to combustion, assuming that we can keep volume constant. This leaves 2 eq, 2 unknowns. (n_2, n_4)

But if we assume no Nitrogen in the Compound then $b = 0$.

Therefore $n_4 = \frac{2(3.76)}{2} n_0 = 3.76\, n_0$

Nitrogen in combustion should be proportional to n_0 (ie combination of O_2 & N_2)

I think that we should be able to apply the method as an alternative to mass changes.

The would be powerful to provide a duplicate method of C_xH_y (and maybe O_2) ratio determinations.

Note $C_xH_yS_aO_zN_b$

does not have to be a hydrocarbon eg C_xH_y or Sucrose $C_xH_yO_z$

It can also be an alcohol for example C_2H_5OH Ethanol

which you might determine as C_2H_6O for example

With no Sulfur!

- Complete combustion: CO_2, H_2O & N_2
- Incomplete Combustion: CO_2, H_2O, N_2, CO, NO_x
- Combustion w/ excess Oxygen CO_2, H_2O, N_2, O_2 (This is us!)

Is this by weight or volume? It is by volume!

There is another really interesting situation. Given: (This is by mass, not by moles!) This adds up to 100% No H_2O?

Combustion exhaust w/ 9.1% CO_2, 8.9% CO, 82% N & no O_2

Assume here, for the problem, that we have C_xH_y — There are NOT MOLES.

$C_xH_y + a(O_2 + 3.76N_2) \rightarrow 9.1CO_2 + 8.9CO + bH_2O + 82N_2$

Solve for n=? m=? a=? b=? ½(y)

Note: Symbols are not clear as to whether "+" is indicated. Seems like it should be.

You are solving for the fuel here.

So there is where it gets very interesting. A Combustion Analyzer will assume the fuel type & then measure CO & O_2 commonly. It will also usually compute CO_2. What it does not do is measure H_2O or N_2

So the Combustion Analyzer is going to give:

$C_xH_y + A(O_2 + 3.76N_2) \rightarrow B \cdot CO_2 + C \cdot CO + DH_2O + EN_2$

These are mass percentages given, not mole percentages. Assume Total weights 100 gms. But then again, how does water fit in to something that weighs is 100%???

We can, even w/ the 100% water question, see how the relationships will work, even w/ a combustion analyzer.

Start simple:

$$C_xH_y + n_0(O_2 + 3.76N_2) \rightarrow n_1 CO_2 + n_2 H_2O + n_3 N_2$$

C: $x = n_1$

H: $y = 2n_2$

O: $2n_0 = 2n_1 + n_2 + 2n_3$

Now the analyzer will assume a fuel, such as kerosene. It has no single formula!

Natural Gas?
Oil #2
Oil #6
Kerosene
Propane

VECTOR

CH_4 C_3H_8 C_4H_{10}
Natural Gas, Propane Butane, LPG, Light oil
Measure O_2, CO
Compute CO_2

Assum We assume it is propane

$C_3H_8 + n_0(O_2 + 3.76 N_2) \rightarrow n_1 CO_2 + n_2 H_2O + n_4 N_2$

We measure O_2, CO, compute CO_2

So we get a number, like 10% CO_2

Assume Complete Combustion w/ excess O_2: CO_2, H_2O, N_2, O_2

• PTA

$C_xH_y + n_0(O_2 + 3.76 N_2) \rightarrow n_1 CO_2 + n_2 H_2O + n_4 O_2 + n_5 N_2$

New Case

C: $n_1 = X$ (eg 1.5)

H: $y = 2n_2$

O: $2n_0 = 2n_1 + n_2 + 2n_3 + 2n_4$

we will use n_0

in this case n_3 (sulfur $= \emptyset$) so

100 12.5

$O: n_0 = \frac{(2n_1 + n_2 + 2n_4)}{2} = n_1 + n_4 + \frac{n_2}{2}$

Theoretical

$\emptyset$ Air

or $n_4 = n_0 - n_1 - \frac{n_2}{2}$

not n_0

N: $2(3.76) n_0 = 2n_5$

for now

$n_5 = 3.76(n_0)$

• PTA is Percent Theoretical Air expressed as a decimal

$\therefore$ Amt of air actually used relative to stoichiometric value

$\therefore$ n_0 is n_0 input

IF PTA = 1.0

$\xi_0 = n_0 \cdot PTA$

$n_0 = n_1 + \frac{n_2}{2}$

Example: Given C_8H_{18} with PTA = 150% (1.5)

$C_8H_{18} + 18.75 (O_2 + 3.76 N_2) \rightarrow 8 CO_2 + 9 H_2O + 6.25 O_2 + 70.5 N_2$

(12.5 · 1.5)

We get for now w/ 100% Theoretical Air:

Meas n_4

this might be measured zero.

goes all the way to the right!

$C_8H_{18} + 12.5(O_2 + 3.76 N_2) \rightarrow 8 CO_2 + 9 H_2O + (\emptyset) O_2 + 47 N_2$

We are basically measuring CO_2.
This gives us Carbon.

The fuel type will give us the hydrogen.

Example:

Assume fuel is Propane C_3H_8.

Imagine we measure 10% CO_2.

Nitrogen gets along for the ride. O_2 will be given w/ complete combustion. So assume the CO_2 & H_2O output is 100% 100 gms

10% of Volume!

10% CO_2 means ~~10 gms~~ are CO_2

The mass percentage of C in CO_2 is:

$12/(12+32) = 27.3\%$

This means $.273(10\text{ gms}) = 2.73\text{ gms}$

$C = \frac{2.73\text{ gms}}{12\text{ gms/mole}} = .2275\text{ moles}$

90% of volume!

So H_2O is ~~90 gms~~ mass % of H is $\frac{2}{18} = 11.1\%$

or $.111(90) = 10\text{ gms of H}$

$= 10\text{ moles of H}$

So if a gas is 10% CO_2 by volume, how much does the CO_2 weigh ---

The molar percent of N_2 in air is 78%
O_2 is 21%

So the Molar percent of CO_2 in our gas is 10%.
the molar percent of H_2O is 90%. NOT TRUE!
no gas are merely correctly. Nitrogen is going along for the ride.

$$C_3H_8 + n_0(O_2 + 3.76N_2) \rightarrow n_1 CO_2 + n_2 H_2O + n_5 N_2$$

$$n_0 = n_1 + \frac{n_2}{2} + n_4 \text{ (!)} \quad n_5 = 3.76 n_0$$

$n_1 = X = 3$
$n_2 = Y/2 = 4$
So
$n_0 = 3 + 2 = 5$ Therefore

$$C_3H_8 + 5(O_2 + 3.76N_2) \rightarrow 3CO_2 + 4H_2O + 18.8N_2$$

I do not think you can solve this w/out an H_2O measurement.
Your results are based upon a false assumption of fuel type so your ratios computed are meaningless!
This is not really your problem. It is

$$CH_y + n_0(O_2 + 3.76N_2) \rightarrow n_1 CO_2 + n_2(H_2O) + 3.76 n_0 N_2$$

but the only number you know is n_1 in mole percentage.
Theoretically you know O_2 (or NO also, however.)
So it should work.

Assumed Values

Assume 2% O_2 & 10% CO_2

$$C H_y + n_0(O_2 + 3.76N_2) \rightarrow n_1 CO_2 + n_2 H_2O + 3.76 n_0 N_2 + n_4 O_2$$

Assume 10% by molar volume CO_2

$$C H_y + n_0(O_2 + 3.76N_2) \rightarrow 0.10 CO_2 + n_2 H_2O + 3.76 n_0 N_2 + n_4 O_2$$

Now we know the relations

$$n_0 = n_1 + \frac{n_2}{2} + n_4 \quad (1) \qquad \text{and } n_1 = 0.10$$

$$n_2 = \frac{y}{2}$$

$$n_2 = (n_0 - n_1 - n_4) \cdot 2$$

This is true but you do not know n_0!!!

You need to know how much water. Then is the original problem.

(n_1) (n_4)

So

$$C H_y + n_0(O_2 + 3.76N_2) \rightarrow 0.10 CO_2 + n_2 H_2O + .02 O_2 + 3.76 N_2$$

n_0

$n_0 = n_1 + n_4 + \frac{n_2}{2}$ ~~This makes no sense~~

$n_2 = (n_0 - n_1 - n_4) 2$ $2n_0 = \dots$ (n_1)

So you still have a problem. You still need to know the amount of water in the exhaust gas. This is the original problem as somehow you need to tie it to H and you cannot w/the combustion analyzer. Maybe the GC but not the analyzer for combustion yet.

You have n_1 & n_4 by measurement.
You do not have n_0 so you cannot deduce n_2
What if I went .21 n_0 mols O_2?
Notice input is atmosphere oxygen. We should reasonably be able to presume
(n_0) (n_1)

$$CH_y + 0.21(O_2 + 3.76N_2) \rightarrow 0.10(CO_2) + n_2(H_2O) + .02(H_2O) + .78(N_2)$$

Therefore $n_2 = (n_0 - n_1 - n_4)2 = (.21 - .10 - .02)2 = 0.18$

and $y = 2 \cdot n_2$ so $y = 0.36$
Si empirical would be
$C_x H_{0.36}$ or $\sim C_{10}H_{3.6}$ This looks great

or $C_1H_{3.6}$ or very close to C_3H_8 which is propane!

Assumed values seem to demonstrate a very realistic scenario.

You therefore seem to have a method which can work. This seems to be quite clever w/ the use of an indirect method w/ the use of a combustion analyzer.

It is dependent upon having both CO_2 & O_2 measurements but not CO

With the IEW CTS Analyzer Model the Accuracy & Range columns are swapped

It is actually measuring

O_2 0-21%

and CO in PPM 0-1000 ppm (0-.1%)

It is not measuring CO_2 directly (0-30%)
It is computing it by the type of fuel.
This might be a problem.

It assumes it knows the fuel H_2 & H_2O(?)

CO_2 is calculated as $\left(\frac{20.9 - O_{2m}}{20.9}\right) \cdot K2$

K_2 is the max theoretical CO_2 in the fuel
O_{2m} is the % oxygen in the flue gas

Example: if we are reading 10% O_2 then we have

$$CO_2 = \frac{20.9 - 10\,(K2)}{20.9} = .5215 \cdot K2$$

if $CO_2 = 10\%$

then $.10 = .5215\,K2$

$K2 = .192$

or 19%

by volume

The says that the max theoretical CO_2 in a given fuel type (NOT OUR FUEL TYPE!) is 19%.

The appears to be meaningless to us. The does not say anything about what the actual CO_2 value is.

"Each fuel has a max possible CO_2 level ($CO_{2\,max}$) which is determined by the fuel composition"
For Natural gas it is 11.8% by volume.
It is never reached in practice. (It would only be reached if the O_2 reading is zero which would indicate perfect combustion)
We do not know the value for our unknown fuel.

There is potentially a serious problem. You are not actually setting a CO_2 direct reading!
Interestingly enough GC will be able to give it to you.

But the theoretical yield is equivalent to n_1 in moles

$$C_xH_y + n_0(O_2 + 3.76N_2) \Rightarrow n_1(CO_2) + n_2(H_2O) + n_4(O_2) + 3.76n_0N_2$$

which is equal to X

So with how a potential lay problem like w/a Calculated CO_2 value.

Original relation:

$$C_x H_y + n_0(O_2 + 3.76N_2) \rightarrow n_1 CO_2 + n_2 H_2O + n_4 O_2 + 3.76 n_0 N_2$$

with $n_0 = n_1 + n_4 + \frac{n_2}{2}$

$n_0 - n_1 - n_4 = \frac{n_2}{2}$

$-n_1 = \frac{n_2}{2} - n_0 + n_4$

$n_1 = n_0 - n_4 - \frac{n_2}{2}$

$n_1 = X$

$n_2 = y/2 = 2(n_0 - n_1 - n_4)$

n_4 = measured or $= n_0 - n_1 - \frac{n_2}{2}$

$n_5 = 3.76 n_0$

$-\frac{n_2}{2} = n_4 - n_0 - n_1$

we know $n_0 = 0.21$

we measure n_4 directly

we know $n_5 = 3.76 n_0$

$\frac{n_2}{2} = n_0 + n_1 - n_4$

Now, the analyzer computes CO_2 as (ratio is over n_1)

$\left(\frac{20.9 - n_4}{20.9}\right) \cdot K2$ ... K2 is theoretical yield.

But we know that $K_2 = n_1 = X$

So what the equation is saying is that "K2" = n_1

$\%CO_2 = \left(\frac{20.9 - n_4}{20.9}\right) \cdot$ "K2" $= n_1 = X \left(\frac{20.9-n_4}{20.9}\right)$

Example: If $n_4 = 0\%$, then "K2" $= n_1$ $K_2 = \frac{20.9 \cdot n_1}{(20.9 - n_4)}$

If $n_4 = 1\%$ then $K2 = 1.05 n_1$

$n_4 = 2\%$ then $K_2 = 1.106 n_1$

$n_4 = 3\%$ $K_2 = 1.168 n_1$ etc

$n_4 = 10\%$ $K_2 = 1.917 n_1$

You can, therefore, express n_1 in terms of n_4 and it will depend on the fuel type. Every fuel type will generate a different O_2 under the same condition. So we can get it back since we know n_4.
BUT WE STILL DO NOT KNOW WHAT n_1 IS !!!

This does indeed create a problem since K_2 depends on the carbon number, which is what we are trying to find out!

So we can determine a certain K_2 ASSUMING that it is a certain fuel type. IT IS NOT THIS FUEL, however. It is something different.

n_4 $f(n_4) \cdot n_1$

n_4 $f(n_4^2) \cdot n_4$

$n_1 = n_0 - n_4 - \frac{n_2}{2}$

known (21) need 0!

$f(n_4^2) \cdot n_1 = n_4$

Two equations Divide:

$\frac{1}{f(n_4^2)} = \frac{n_0 - n_4 - \frac{n_2}{2}}{n_4}$

This leads to: (known) n_4

$$f(n_4^2) = \frac{n_4}{n_0 - n_4 - \frac{n_2}{2}}$$

(known) (known) (known) (unknown)

$$\frac{1}{f(n_4^2)} = \frac{n_0 - n_4 - \frac{n_2}{2}}{n_4}$$

$$\frac{n_4}{f(n_4^2)} = n_0 - n_4 - \frac{n_2}{2}$$

$$-\frac{n_2}{2} = n_4 - n_0 + \frac{n_4}{f(n_4^2)}$$

$$-n_2 = 2\left(n_4 - n_0 + \frac{n_4}{f(n_4^2)}\right)$$

or (known) (known) (known)

$$n_2 = -2\left[n_4 - n_0 + \frac{n_4}{f(n_4^2)}\right]$$ we must now try to solve this.

and then $n_4 = n_0 - n_1 - \frac{n_2}{2}$ or $-n_1 = n_4 - n_0 + \frac{n_2}{2}$

and (known) (known) (solved)

$$n_1 = n_0 - n_4 - \frac{n_2}{2}$$

Now back to:

$\%CO_2 = n_1 = \frac{20.9 - n_4(100) \cdot \text{"}K_2\text{"}}{20.9}$ $\quad K_2 = \frac{20.9 \cdot n_1}{20.9 - n_4(100)}$

$n_4(\%O_2 \cdot 100)$	" "
0%	then $K_2 = n_1$
1%	$= 1.05 n_1$
2%	$= 1.106 n_1$
3%	$= 1.168 n_1$
10%	$= 1.917 n_1$

Therefore

$n_4(O_2)$	" "
0	then "K2" $= n_1$
.01	$= 1.05 n_1$
.02	$= 1.106 n_1$
.03	$= 1.168 n_1$
.10	$= 1.917 n_1$

" $K2\text{"} \cong 50.45 n_4^2 + 4.11 n_4 + 1.00$

$r^2 = 1.000$

$f(n_4) = f(n_1)$

" $K2\text{"} = f(n_4) \cdot n_1$ So if you read CO_2, you should be able to back at a function of n_1.

Example ~~$n_4 = 3\%\ O_2$, or 0.03~~ ~~then~~ If you read ~~7% CO_2 or more, then~~ 3% O_2 (=.03) then $K_2 = 1.169 n_1$

Therefore we know that

$\%CO_2 = \left(\frac{20.9 - 3}{20.9}\right) 1.169 n_1 = 1.00 \cdot n_1 = n_1$

which we know to be true, ie $n_1 = X$

Ok, $\%CO_2$ will always be equal to n_1

and it does not matter what the fuel type is

to now she is very interesting. It seems as though you have what you need.

Jun 29 2016

We now have an oxygen concentration meter coming from China for $80. This is going to make life much easier and will be a tremendous trouble to the GC-TDC to separate oxygen from nitrogen.

One lesson learned here is that oxygen concentration as a function of altitude is a very significant factor to keep in mind.

O_2 meter will measure O_2 pre & post combustion.
GC-TDC will measure CO_2 easily, even CO if it exists and $(N_2 + O_2)$ combined.
H_2O may also measure water vapor. We should be fairly well equipped now for CH ratio analysis but two different methods. (H_2O - tailing factor)
1. Chemical - mass analysis
2. GC-TCD combined w/ O_2 measurement, & ability to separate from N_2.

You can now review the analytical methods that are in place. You can also bring CO into the picture as well as consideration of O, N & S added to the organic compound.

The next most interesting case is

$$C_xH_yO_z + n_0(O_2 + 3.76N_2) \rightarrow n_1CO_2 + n_2H_2O + n_4O_2 + n_5N_2$$

Carrying Forward:

$$C_{n_1}H_{2n_2}O_z + n_0(O_2 + 3.76N_2) \rightarrow n_1CO_2 + n_2H_2O + n_4O_2 + n_5N_2$$

We know that — 5 unknowns: n_0, n_1, n_2, n_4, n_5

C: $n_1 = x$

H: $2n_2 = y$

O: $z + 2n_0 = 2n_1 + n_2 + 2n_4$

or $z = 2n_1 + n_2 + 2n_4 - 2n_0$

also we know

$3.76\,n_0 = n_5$

Lets take sucrose $C_{12}H_{22}O_{11}$ MW = 342.3

$x = 12 = n_1$

$y = 22 = 2n_2 \Rightarrow n_2 = 11$

We know @ sea level. $n_0 = 0.21$ Molar Concentration (gas)

We need to be able to convert between molar concentration & grams. 12 moles

We now have M

$$C_{12}H_{22}O_{11} + [0.21](O_2 + 3.76N_2) \rightarrow 12CO_2 + 11H_2O + n_4O_2 + [0.79]N_2$$

M

O: $12 + 2[0.21] = 2(12) + (11) + 2n_4$ — with complete combustion, n_4 is zero!

$2n_4 = 12 - 2(12) - 11 + 2[0.21]$

M

So the question is what does [0.21] actually mean here?

Remember 150% = 1.5 ?

Excess Air?

SPONCH covers most everything organic
(phosphorus / sulfur / oxygen / nitrogen / Carbon / hydrogen)

Another thing to notice. If molar concentrations are in %, then the total should add up to 100.

You have an issue here of some units in gas and some units in %.

12 moles + 11 moles + N_4 moles + 79%
1 mole of sugar → 12 moles CO_2
1 mole of sugar → 11 moles H_2O
Nitrogen is going along for the ride. It is not involved.

Use fundamental balancing law

$11 + 2 \cdot n_O = 2(12) + 11 + (N_4)$ but under complete combustion N_4 may be = to 0!
or
$2n_O = 24 + 11 - 11 \Rightarrow 2n_O = 24$ or $n_O = 12$
So now, under conditions of complete combustion, we do know n_O
$n_O = 12$ moles.

So the upshot of this is always shoot for complete combustion if at all possible.

So now we have.

$$C_{12}H_{22}O_{11} + 12O_2 + 45.12N_2 \rightarrow 12CO_2 + 11H_2O + (\emptyset)O_2 + 45.12N_2$$

(Theoretical)

These two values determined (even in %) will give you the C-H ratio.
They will also tell you the total oxygen (contained & utilized together)

In the process of combustion
eg $(z + C = 24 + 11 = 35)$
(a, b, c)
sulfur, Nitrogen

Rule of 13.
can also be used to assist in determination of no. of hydrogens.
(gas tube spectroscopy)

You have some very valuable methods that have been developed here for fundamental organic analyses & elemental analyses

1. Combustion – mass – stoichiometry
2. Combustion – GC – TCD – O_2 sensor
3. Balancing of combustion reactions

Rule of 13:

(12) + (1)

every C has a hydrogen

1. MW ... 12, divide MW by 13

13

eg MW = 164

MW = 12 + 8 remainder

13 (C–H combinations)

This part alone would yield $C_{12}H_{12}$ + 8H ⇒ $C_{12}H_{20}$ [3] (Unsaturation)

This is the first estimate MW = 164

Next, if the saturation variants, we design substituting an oxygen

1st oxygen goes to $C_{11}H_{16}O$ [4] V: $C_{11}H_xO$ ⇒ 148

and then $C_{10}H_{12}O_2$ [5] 164 − 148 = 16

$C_9H_8O_3$ [6] so $C_{11}H_{16}O$

$C_{10}H_xO_2$ ⇒ 152

etc so $C_{10}H_{12}O_2$ 164 − 152 = 12

Some of the substitution processes are:

O for CH_4 ... 16 (AMU) ... How does each affect the degree of unsaturation

N for CH_2 ... 14 (AMU)

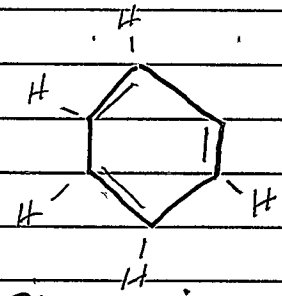
H_{12} for C ... (12 AMU)

You ask if the structure make sense, especially of respect to saturation levels.

A degree of unsaturation often or usually means a phenyl ring structure.

Now start looking @ the spectra, esp IR & NMR. Very cool work here.

A phenyl ring is C_6H_5



Phenyl ring
It is a benzene ring without one hydrogen

By the degree of unsaturation is equal to the sum of double bonds + no. of rings

So benzene has 4 degrees of unsaturation.

3 double bonds + 1 ring = 4

Lets see if we can figure out why

Alumina is more polar than silane

Alumina is more acidic (reacts w/ base) than silica oxide (more alkaline & used therefore react w/ acids)

Lets start w/ electronegativity:

		Δ
Al	1.61	1.83
Si	1.90	1.54
O	3.44	

Now that is interesting. I would have expected an alumina column to be more polar than a silica gel column. But apparently the reverse is true. Why?

Ok, guess what? We have found a source that says exactly what I am saying.

It states silica gel is less polar than alumina

This makes sense to me.

It also says that silica gel is an acidic adsorbent, & thus preferentially retains basic compounds.

Another source says that "aluminum oxide is more polar than silica gel" which also is making sense to me.

Therefore alumina is a rather polar column. Knowing this, you can now consider how the analyte and the eluent (mobile phase) interacts with this type of column.

Let's take the question first and then deal w/acidity or alkalinity.

" . "

A strong eluent will therefore be polar for an alumina column. Water is an extreme example, therefore. This water will then interact "strongly" with the polar column and "blanket" it, thereby preventing or reducing analyte reaction with the column. Non polar or less polar analytes then would therefore be expected to elute from the column very quickly, which is certainly what we have a case of with the HEPA 1st elute.

Now we know we have a second compound that very much adheres to the column more closely. This indicates that it is of a more strongly polar character. We know that this is also true because we know that it is a highly water soluble protein.

You have already, therefore, an important predictive distinction between the two elutions.

Now, out of curiosity, what does the IR show @ this point? Do we have this information with us?

Based upon Jun 16 notes a IR plot, we clearly show the situation. The opaque first HEPA elute has alkane & amine as dominant proteins. It may n may not have an aldehyde within it. As I recall, this plot was difficult to obtain because of the low concentration & high water concentration output from the column using water as the eluent. But clearly the result fits quite well, alkane and amines portray a definite species of interest that is of a less polar nature than that which follows.

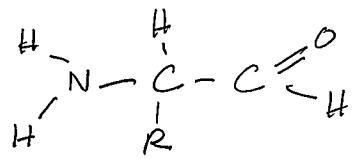
C-H N-H
Bands dominate w/ the 1st HEPA opaque elution.

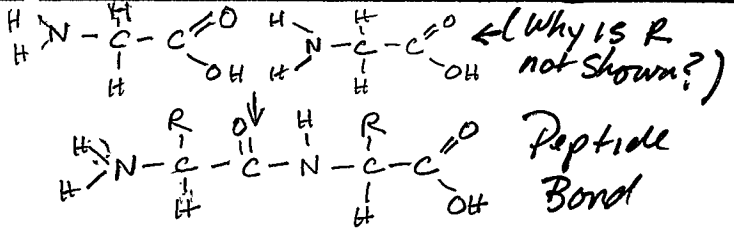
Getting the molecular weight n C-H ratio and nitrogen would be of much interest here. This would be difficult w/ such a weak concentration.

We also verified amines in Jun 16 w/ Ninhydrin.

We do not verify a protein, we do verify an amine.

Amino Acid Structure:





(Why is R not shown?)

Page 88

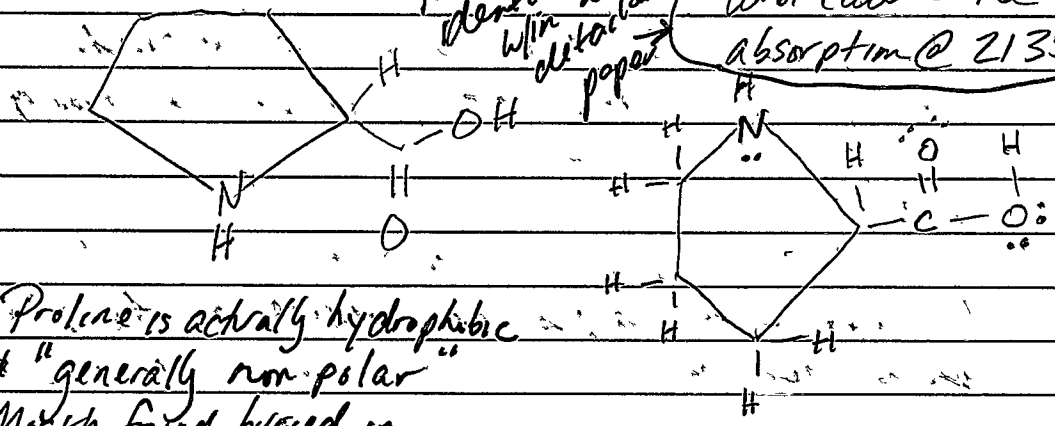
Now, the second elute, which we know to be a protein from the HEPA filter, should be, by LC analysis, be of a more polar nature.

What does IR say here? Do we have the spectrum? Yes we do, also from Jun 16. We also know the elute (colored / protein verified) is strongly acidic.

The IR plot strongly shows the presence of
1. Acid
2. Aromatics
3. Alkynes - Proline issue?

Prospect: a proline rich, aromatic, acidic protein.
Tryptophan, proline, glutamic acid

Proline is unique, it is an Imino acid, vs an amino acid. Imino acids are used for biosynthesis.



Proline is actually hydrophobic & "generally non-polar" Mostly found buried in the protein.

~~Definitely a polar~~ ?
2. Definitely is acidic

The side chain is the ring.
This is generally non-polar

Every amino acid has an acid group & polar OH

No one seems to be talking about the strong absorption of proline rich proteins, eg. saliva, @ ~ 2135 cm^{-1}

Why?

" "

Statherine is a representative proline rich protein. It is a salivary protein.

The paper (pdf, a little hard to get)

"Body Fluids & Spectroscopic Techniques in Forensics: A Perfect Match"

Journal of Forensic Medicine. 2015

has the IR spectra of interest for saliva that shows the strong absorbance @ ~ 2135 cm^{-1}.

Collagen and elastin are noticeably rich in proline. Important for proper functioning of joints & tendons.

Proline is synthesized from glutamic acid.

Collagen is the main supportive protein of skin, tendons, bones & connective tissue.

$C_5H_9NO_2$

Highly soluble in water & alcohol,
Slightly soluble in ethanol & acetone.
Insoluble in ether, propanol.

It appears to have significant optical rotation (~ -82° !)

Maintains & strengthens heart muscles.

Only sometimes called an "imino acid".
The IUPAC definition of an imine requires a
Carbon nitrogen double bond.
Associated Disorders & Diseases:
Tied in w/ Alzheimers disease

Proline Rich Polypeptides - reduces Alzheimers

Tau Proteins - folding disruption of proteins -
Proline - Alzheimers
Tau is a highly soluble protein
Unusually hydrophilic
proline rich domain
These proline rich areas within the Tau protein
are diagnostic of the disease state.

Proline mutations

Jun 30 2017

Now let's look @ acidity & basicity of alumina & silica gel and the impact of this upon column behavior

Alumina: Acidic or Basic and why?

"Aluminum oxide displays acidic properties"

"Preferential adsorption of acidic substances on alumina..."

Basic Oxides ← → Acidic Oxides

Na_2O MgO Al_2O_3 SiO_2 P_4O_{10} SO_3 Cl_2O_7
P_4O_6 SO_2 Cl_2O

Al_2O_3 is considered amphoteric and in the "middle" of the series. Notice it sits both ways & that SiO_2 is even more acidic.

Silica gel is SiO_2. It is of a porous nature
Alumina is Al_2O_3

Start w/ Na_2O. Why is it basic?

One answer given is:

$Na_2O \rightarrow 2Na^+ + O_2^-$
$O_2^- + H_2O \rightarrow 2OH^-$] 2 step process

Called a metal hydrite.

Another answer given is:

$Na_2O + 2H_2O \rightarrow 2NaOH + 2H_2O$

Another source says that "alumina is more basic than silica gel".

& therefore we see again, as often happens, that answers vary widely on such topics.

A Chromatography site states that "Silica and alumina are most polar adsorbents so polar components (this we know must include both the analyte and the eluant) in the mixture are retained more strongly on the stationary phase and are therefore eluted from the column last. Silica is recommended for most compounds, but as it is slightly acidic (ok, this matches) it preferentially retains basic compounds. Alumina is slightly basic so it will retain acidic compounds more strongly. It is good for separation of components that are weakly & moderately polar and the purification of amines."

Everything in the statement appears to be accurate so it will be accepted @ this point.

* We still do not know why SiO_2 is more acidic than Al_2O_3 or visa versa, why Al_2O_3 is more basic than SiO_2.

No one really seems to be answering the question.

Along the way, however, a Company article clearly proclaims the marked superiority of alumina over silica gel in chromatography. (Dynamic Adsorbents)

The primary reason is for the amphoteric properties of alumina (outlined earlier) and for its temperature & pH stability.
The article seems quite sound and silica gel does seem prone to a host of problems (esp pH) that will degradate & even dissolve the stationary phase.

We may already be in the best position possible w/ the column that has been constructed and that is already performing quite well.

We may eventually be forced to use the second column w/ alumina also (not as likely to be used in other capacity) or use silica gel understanding the limitations, esp w/ regard to pH & limited ion exchange capabilities.

Silica gel pH < 4.5 causes problems
Also high pH causes problems.
Phosphate & Carbonate ions cause problems also.
Silica gel also can apparently dissolve in water or polar solvents so the sources like it could be very problematic. "Dry loading" might be required.

Jul 01 2017

The project list can be restated

1. DNA production of CDB (volume increased) and laboratory coordination
2. Continue to nail down nature and extent of the identified protein(s)
3. Continue work w/ elemental analyses & molecular weight determination. GC & O_2 meter will come into play. Specific heat relationships.
4. Cetegen samples
5. ICMP release
6. Courses: Organic, Davis - Gen Chem & Biol - Chem
7. Monitor cultures
8. UV software purchase?
9. Holmes work continued
10. Microwave digestion trials
11. Brain wave study
12. Electrochemistry

Jul 03 2017

A bit of electrochemistry today & voltammetry.

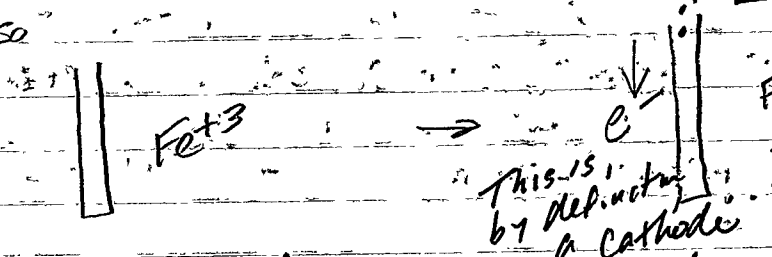
Chemists define the electrode @ which reduction occurs as the cathode.

Chemists define the electrode @ which oxidation takes place as the anode.

These are the definitions. Now you interpret them.

Reduction occurs when the oxidation state is reduced, eg $Fe^{+3} \rightarrow Fe^{+2}$ or $O^{-4} \rightarrow O^{-2}$. This means that electrons are gained @ that electrode.

Positive no. + (−) → Less Positive



means that electrons are pumped into the solution.

We know that in a traditional battery that current (ie, electron) actually flow from the negative terminal towards the positive terminal.

They say to me that the negative terminal of a battery is the cathode. Is that true?

And no wonder this is confusing, there is good cause for it.

A "conventional current" describes the direction in which POSITIVE !!! electronic charges move.

Electrons have a negative charge, so the movement of electrons is OPPOSITE to "conventional" current flow.

So you can see why Chemistry use the definitions that they do. "Conventional current" vs actual electron flow is quite a mess, but the terminals @ which reduction and oxidation occur, the service they involve electron transfer, are FIXED by definition. With "conventional current" the terminals change dependent upon whether a battery is discharging or charging! Speaking in term of "conventional current" flow certainly introduces confusion into the matter since it is the opposite of actual electron flow. No wonder it is confusing.

Now lets go back to our opening in the India electrochemistry video.

Before this: "the flow of electrons is almost always from anode to cathode outside of the cell in device and operating mode"

— yes, this makes sense

Now our Indian video says exactly what we have just established:

The site @ which reduction occurs is the = cathode.

The site @ which oxidation takes place is the anode.

This is all as it is supposed to be. The chemical definitions of cathode (reduction) & anode (oxidation) are unambiguous.

The reason for example, you should be able to look @ the hydrolysis of water and determine which is the cathode & which is the anode by inspection.

(reduction) @ the cathode: $2H^+(aq) + 2e^- \rightarrow H_2(g)$ $E = 0.00V$

@ the anode (oxidation): $2H_2O(l) \rightarrow O_2(g) + 4H^+(aq) + 4e^-$ $E = -1.23V$

Now to balance this, we have

$4H^+(aq) + 4e^- + 2H_2O(l) \rightarrow 2H_2(g) + O_2(g) + 4H^+_{(aq)} + 4e^-$

$2(4(H)\ 2(-2))$

$2H_2O(l) \rightarrow 2H_2(g) + O_2$ ie hydrogen is reduced

oxygen is oxidized.

Therefore we know that

1. Hydrogen is being reduced.
2. Hydrogen is therefore being produced @ the cathode
3. Hydrogen is being produced @ twice the volume of oxygen.

The half reactions are not all that obvious. In the figure case, we see that

1. Hydrogen ions are being reduced to produce hydrogen gas (oxidation state goes from +1 to 0). in half reaction #2, we see that

2. Water is being split into a gas (Oxygen) and an ion (H^+) and the oxygen is being oxidized in the process. (oxidation state goes from −2 to 0 in the process)

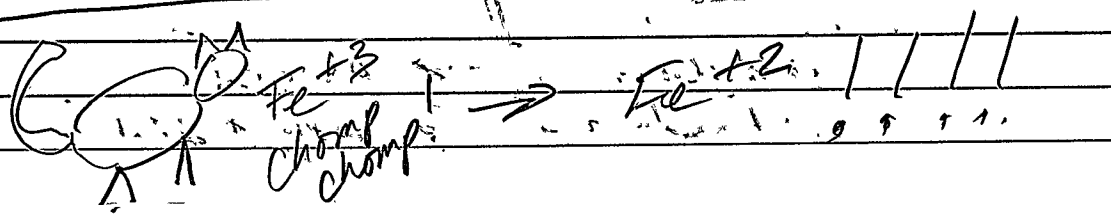
You can always tell now by direct observation & deduction of current flow (ie electron flow) which is the cathode & which is the anode.

What will be your mnemonic for always recalling reduction ~~that oxidation~~ take place @ the cathode and Oxidation ~~reduction @ the~~ anode? (See! Confusion already)

R-C REDUCTION - CATHODE
O-A OXIDATION - ANODE

Any species gets smaller when it encounters the CATHODE (because CAT eats it up and makes reduce it size!)

So Reduction takes place AT the CATHODE.



Ok, these are the basics. Just they are @ the beginning of everything.

You have remained unclear in differential voltammetry which species has been oxidized & which has been reduced. You are on the path toward eliminating that confusion.

Indian video (Indian Institute of Science) provide a wonderful discussion of electrodes that make this process clear for the 1st time. Electrodes of the 1st, 2nd & 3rd kind. We now understand that we are using electrodes of the 3rd kind, which seems like a great setup, as the electrode (Carbon in our case) is inert.

1st: a metal in an ionic solution of the very same metal.
eg $Cu^{2+} + 2e^- \leftrightarrows Cu_{(s)}$
ie, A Copper rod in a Copper ionic solution.

2nd: A metal in a salt solution of that same metal, eg Ag rod in a silver Chloride solution.

A mercury rod (drop) in a mercuric salt solution

eg $AgCl + e^- \leftrightarrows Ag + Cl^-$ $E_0 = +.222V$
$HgCl_2 + e^- \leftrightarrows 2Hg + 2Cl^-$ $E_0 = +.268V$

These are very reproducible cell types so they an advantage! They can therefore be used instead of a "standard hydrogen electrode", (which is difficult in practice).

We can go to another step:

(Standard Calomel Electrode)

$Hg_2Cl_2 + 2e^- \rightleftharpoons 2Hg + 2Cl^-$ $E_0 = 0.246V$ SCE

(In saturated KCl solution)

vs (Standard Hydrogen Electrode)

$Hg_2Cl_2 + 2e^- \rightleftharpoons 2Hg + 2Cl^-$ $E_0 = +.280V$ NCE

$AgCl + e^- \rightleftharpoons Ag + Cl^-$ $E = +0.237V$

(in KCl solution)

3rd: Oxidizing + Reducing species Stated are both in solution w/ an INERT electrode (eg platinum, carbon, etc)

eg $Fe^{3+} + e^- \rightleftharpoons Fe^{2+}$ $E_0 = +0.771V$

$2Hg^{2+} + 2e^- \rightleftharpoons Hg_2^{2+}$ $E_0 = +.920$

The only function of the electrode is to transport electrons to the ions. The electrode is not involved in the reaction. This is what we are using and now we understand why Carbon works — it is inert.

It is great to understand for the first time

The convention is that you write the oxidized species on the left of the equation and the reduced species on the right side of the equation.

E_0 of Cu refers to a Cu rod placed in an ionic copper solution of (same metal, same ion)

$Cu^{2+} + 2e^- \leftrightarrows Cu \quad E^\circ = -0.337V$

w/ platinum(2) or 2 carbon electrodes.

You can test this for yourself any time you feel like it and you can measure it.

Try this sometime!

$Zn^{2+} + 2e^- \leftrightarrows Zn \quad E_0 = -.763V$

$2H^+ + 2e^- \leftrightarrows H_2 \quad E_0 = 0.00V$

These are observed values

You can flip the current to test if the reaction is reversible.

Methods: We will cover the first two.

Polarization

Overvoltage

* A. Polarography

* 1. Potentiometry - defined

2. Voltammetry - defined

3. Amperometry

4. Conductivity

4. Oscillometry

5. Coulometry

6. Chronopotentiometry

The distinction between electrodes of the 1st, 2nd & 3rd kind I find to be very helpful. It lets us know when we stand w/ some graphite electrode.

I can also use the EIS, electrical impedance spectroscopy entices me a great deal. It is very tangible in the outcome and interpretation as an electrical circuit counterpart.

- With more conventional approaches, I have the strongest interest w/ Normal Pulse Voltammetry (Derivative) and AC voltammetry (derivative also, as I recall).

Jul 07 2017

Time to get the GC running again..
Start at room air.
Leak check at the column fittings.
Remove the sleeve from the 5ml syringe
w/ the dremel tool - it will help matters.
We are going to try to constrain the GC
to gases as much as possible. We see that
water & alcohol cause a lot of problems for
stability w/ the column.
There is some residue in the column, I have to
think that it is water.

It looks like flow rate is set to 6 ml/min.
* Detector is set to 225°C
* Oven is now set to 180°C
* Oven max is set to 230°C..
These are probably reasonable numbers.
Check the max temp of the column...

Lots of small contaminations, as well as a
couple of large ones, are in the column. Clean
it out.

Bring column up to 200°C
Ops must keep the column clean.
The column is starting to get cleaner after an
hour of baking & conditioning

We do have some information on the new column.
It is definitely a Hayes Sep D column.
It is packed w/ divinyl benzene
Max temp is 290°C which is great.
Polarity is "1" - What does this mean?

We had another extended peak come out - another polar material? The column keeps looking better and better again. Watch the water a polar solvent!

Column up to 220°C. This is about max.

Now combustion gives CO_2 & H_2O, so there is a problem also for the column. You will have to see if you can clean it out of the column.

You might want to try to fill up a balloon ~~as~~ w/ the combustion chamber. Also you need to calibrate basic gases.
The column is slowly getting cleaner. We will just keep working at @ 220°C when we can. Drop down to 200°C now.

There should be water vapor even in human breath. CO_2 level w/ direct breath out is very low.

Really nice CO_2 peak by holding breath @ 1.126 min
You might want to try & capture the CO_2.

	Peak Height	%
$O_2 - NO_2$	334.2	99.24%
CO_2	2.51	0.76%

No Hold: Peak Ht
$N_2 + O_2$ 119.44 99.86%
CO_2 .072 + .096 = .168 Ø.14%

Increase in CO_2 from holdy breath ~45 sec
is $\frac{Ø.76}{Ø.14} \cong 5.5$ times increase

Normal air is about .04% CO_2

$\frac{.04}{100} = \frac{X}{1E6}$ $X \cong 400$ PPM

Not holding we measure ~1400 PPM
Holdy breath we measure ~7600 PPM

$\frac{7600}{400} \cong 19$ time increase

Not holdy breath
$\frac{1400}{400} \cong 3.5$ times increase

One source say that it is 4%, and
we see that we are not even close to
that value even upon holdy my breath.
Why is this?

"Anerobic culture" is producing an interesting result. CO_2 concentration does not look especially high. But we positively have a second gas that has been formed @ 4.30 min. A broad low peak but it is detectable

t.m	Peak Ht		
0.37	$O_2 - N_2$	221.54	99.92%
1.11	CO_2 peak	.102	.05%
4.30	?	.071	.03%

What is the gas? It is labeled as CO.
Test this with vehicle tomorrow.

2nd Culture under pressure showed no $N_2 - O_2$ peak, no CO_2 peak, no 4.30 min peak — how can this be? The make no sense.

Again we have a problem. No air peak - why?

Hold break test: Peaks should up immediately. All is normal here. What happened w/ culture?

Something was in those cultures, what happened?
OK, Now we have it again. Maybe the needle did not plot properly. We lost the $N_2 - O_2$ peak and the CO_2 peak (CO_2 is small).
No CO peak in successful culture. Ya saw the variation before

Jul 05 2007

I made two trial extractions today from plant material. You used leaves from one plant & purple flowers from another. The solvent was water w/ strong lye dissolved, actually KOH. You also heated mixture as digestion and it allegedly can work.

You ate in water the time. You neutralized the pH the time but you could also have shifted the solvate into a more less polar solvent, probably MEK. Then you would not have had to neutralize the pH and you probably would have a more convenient sample, at least if it work. There are many different directions you can go. It would take a lot of work to separate out any components, LC etc so you must choose your samples carefully. I have confidence I can do more with empirical formula, elemental analysis and MW now for most any sample. The sample, however, needs to be important as a great deal of work is involved in the process.

The immediate goal now, however, is to identify the GC peak of carbon monoxide. We will use car exhaust as a reference.

The car exhaust sample did not CO @ the time. You can use a jar and a candle.

Your effort to produce CO appear to be unsuccessful @ this point. The issue may be one of sufficient concentration. Both the culture as well as the exhaust analyses have been variable in producing the gas in question w/ retention time of ~4.30 min @ 80° C on Hayesep D column.

As we look back, the candle method of production seems to be a question of concentration also. I believe the HVAC tests in the gas tube video was measuring approx 10-20 ppm. This is not sufficient for GC TDC. I believe I can detect @ about 100 ppm under good conditions, but not in the teens. The filaments are on high current as well but 20 ppm is simply not sufficient there.

CO Production Methods:

Best (85%) 1. Formic acid + Conc. Sulphuric Acid (98%)
2. Oxalic Acid + Conc. Sulphuric Acid + Heat

Flame test.

Really very good video here by "Chem Player" channel. No formic acid here though.
"Carbon monoxide preparation + how it is deadly"

Page 109

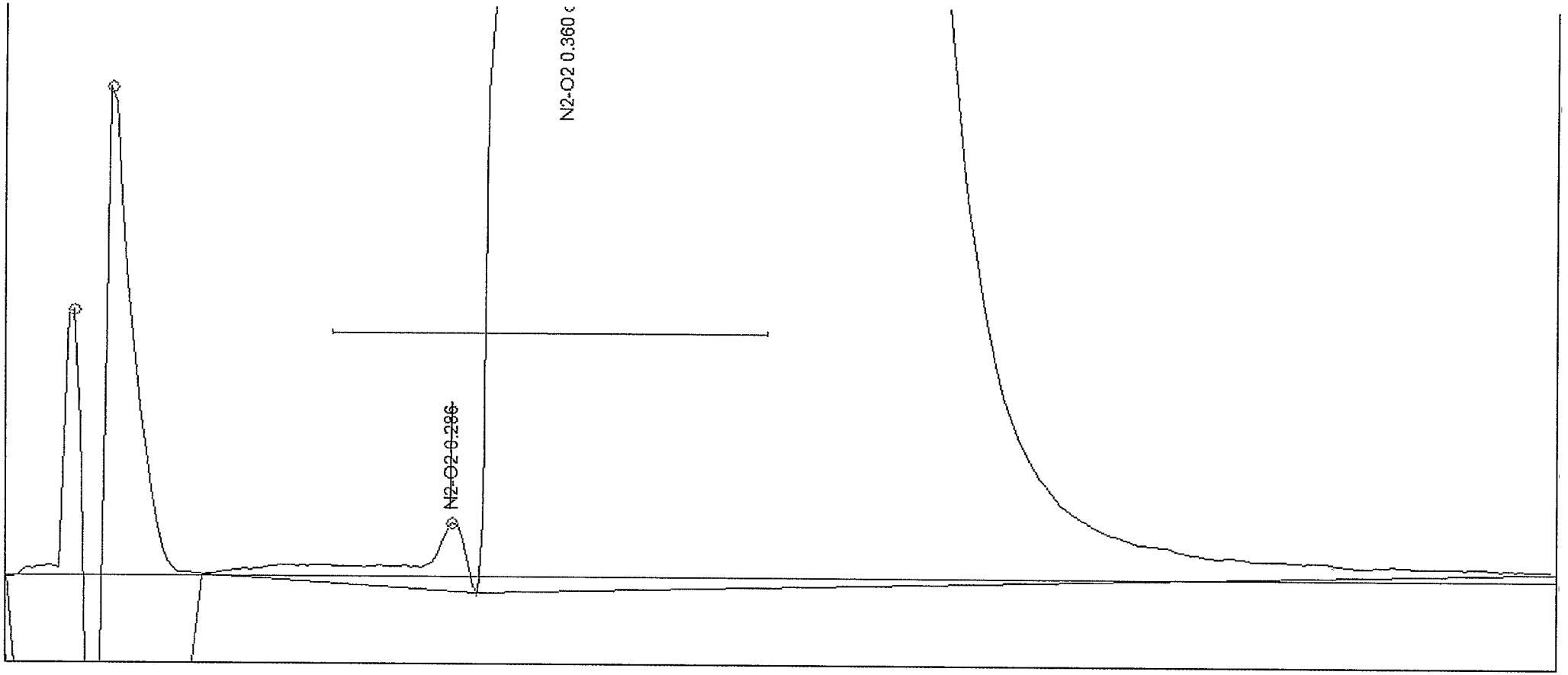
We have some very good success w/ headspace analysis of charcoal powder (briquette). Red Hot sample.

I believe that we have separated, in order: 80°C

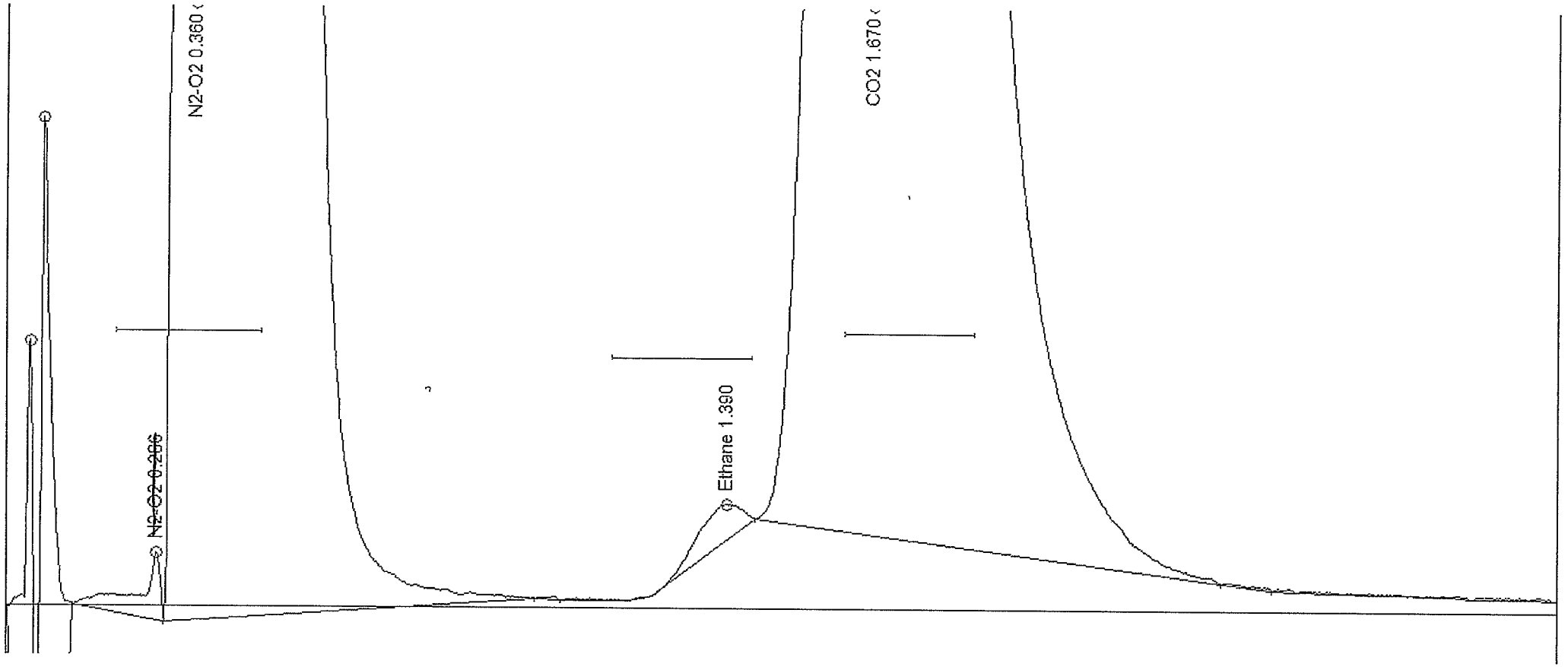
0.28	1. Methane (very close! to O_2 - N_2 peak)
0.36	2. O_2 - N_2 peak
(3) 0.53, 1.39	3 Ethane
1.67	4. CO_2
4.1, 4.5	5. Propane or CO? a bit close maybe CO, then propane
6.10	We have 2 additional peaks
10.51	

Altogether we have 9 peaks to sort out & standardize. One of them is almost certainly be CO. You will be able to distinguish between CO & propane.

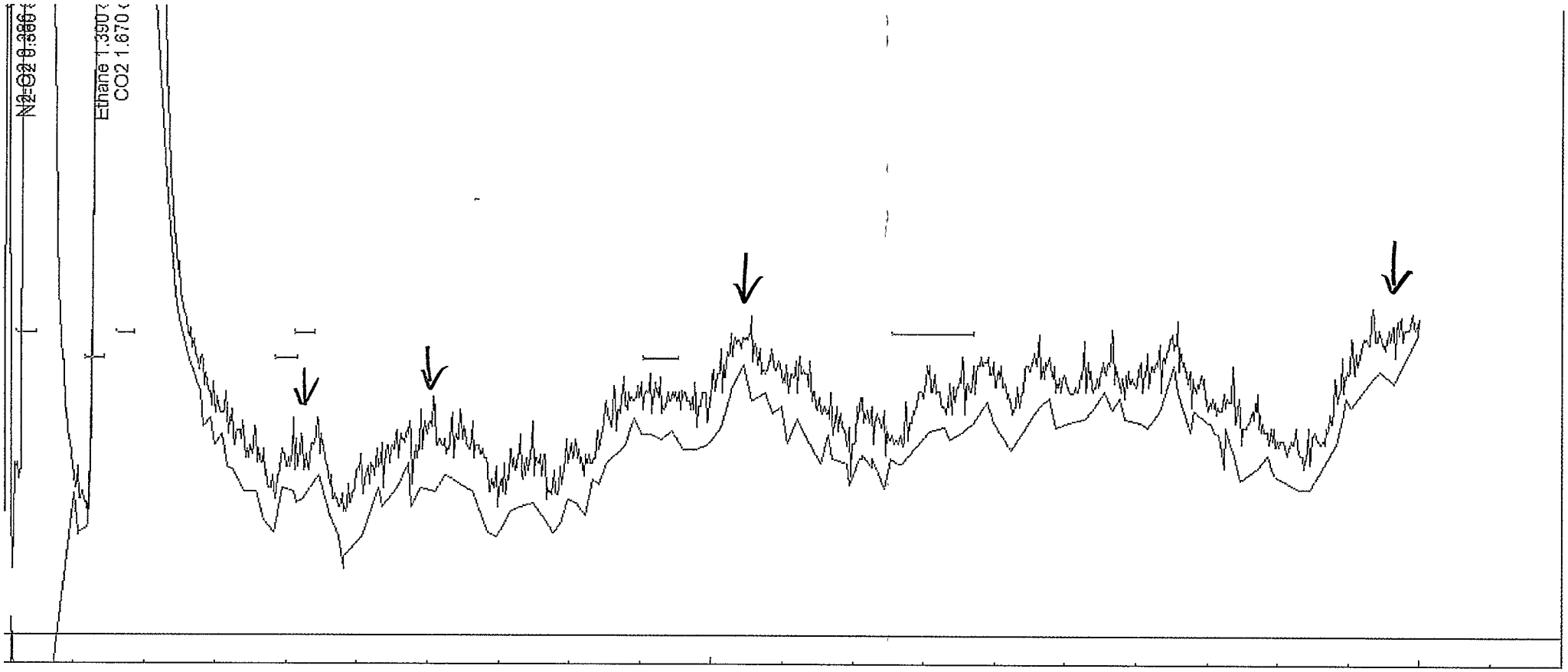
Charcoal Headspace



Charcoal Headspace



Charcoal Headspace



Jul 06 2017

Headspace Analyzer: Distilled water.

There is NO water peak @ 4-5 min with a headspace water analyzer. This tells us that the peak is not water.

Anticipate that water should still be in the column so let bake out and expect a strongly tailing peak.

We next try to settle if the peak is propane or another light hydrocarbon: things a comparison w/ various knowns.

Run the column @ 220°C until you know that it is clear.

I am studying the effect of H_2O, by strictly upon the column. Attempted was H_2O vapor via headspace / heating.

H_2O is behaving in the Hayes Slp D (HSD) column exactly as expected & as I recall. Extremely long tailing only after column brought up to high temp, by 220°C for prolonged time (@ 45 min)

The HSD column actually appears to be reacting essentially identically to the silica gel original column, I see no major difference @ this time.

H_2O in the column, as it has always been, remains a problem and difficult issue. Try to avoid it if @ all possible. It's not.

Now we can move on to propane testing, butane etc to work on the 4-5 min appearance in the column @ 80°C.

Methane	C_1
Ethane	C_2
→ Propane	C_3
Butane	C_4
Pentane	C_5
Hexane	C_6
Heptane	C_7
Octane	C_8

Jul 08 2017

We are looking @ sugar combustion (via the headspace apparatus vs the combustion apparatus) on the GC.

We have made a run @ 80°C & now 220°C
At 80° we have the N_2 - O_2 peak. Also had a defined CO_2 peak. Then upon ramping to 220° appeared to pick up a small peak (?) @ ~ 20 min. Residual H_2O may have been in the column since the run was terminated

A run @ 220 as shown O_2 - N_2 and two major broad tailing peaks. We may have a mixing of runs here. Column need to clear out @ 220° then suggest

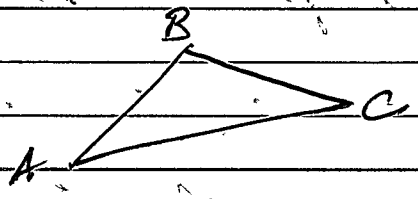
80° for 3 min
Rapid ramp to 220°C
Hold @ 220°C for ~ 20 min.

The goal here is to investigate the GC-TCD for combustion analyses

CO_2 has a response factor of 48 MW CO_2 = ~~34~~ 44
Water has a response factor of 21 MW H_2O = 18

~~48/44~~ 44/48 = 1.069 CO_2 to water
18/21

Area of a triangle by coordinates is



$$\text{Area} = \frac{\left| Ax(By - Cy) + Bx(Cy - Ay) + Cx(Ay - By) \right|}{2}$$

	X	Y	x	y
A	.07	.68	6.22	.698
B	1.72	1.98	7.76	2.45
C	5.84	.66	12.56	.736

Area = ~~1.13/2 = .565~~ 3.23 5.24

OK, then looks decent.

If we apply the CO_2 to water weight factor we have

3.23(1.07) = 3.46 & 5.24 And we know that this is too low.

Run now @ 80°C → immediate ramp to 220°C.

I think I missed the inlet partially

It looks like we may indeed see a CO interrupter right on mark @ 4.31 min.

We now have response factors and weight factor computations with a spreadsheet that will assist.

With the headspace method, it seems as though you need a fresh sample each time.

Injection must also be complete & uniform.

With fresh sugar sample and clean column and all in order we consistently see a very small spike @ the CO point. The molecular sieve and conjecture our desired proposition.

What we are doing now is trying to get a clean read out on H_2O content.

80° → rapid ramp → 220°C

We have

1. O_2-N_2 combined (molecular sieve separate O_2 is cumbersome)
2. CO_2
3. CO (extremely minor but detectable)

You need to run a column with nothing injected but still the same thermal programming. This can be revealing w.r.t. H_2O present.

Notice the inflection point occurring @ 13.05m - 14m. In effect you may have seen the above on a previous run.

It appears in general that the H_2O level might be relatively low. The CO_2 peak is not exactly large either so the could be realistic and superimposed upon the thermal decay function.

There are only clear runs we have obtained the CO level produced must be dramatically low. I wonder if the candle experiment might show replicate that.

Clear runs made. We may have small amt of H_2O coming thru in the sample runs & not the control runs, but if there it is in a small amount.

You are confirming the primary objective. The peak @ ~ 4.3 min does indeed appear & the CO.

Tell the cultures (anaerobic) have a dynamic CO_2 peak.

Jul 10 2014

Model / Theoretical

Tabulate GC data

Butane 9.27 9.150

Ethane 1.356, 1.360 1.326 1.38 1.33 1.34 1.38

Propane (?) 4.23 4.153 4.2

O_2 .386 .363 .353

N_2 .386 .363 .353

Ø.56 Methane Ø.57

CO_2 1.72 1.62 1.64 1.69 1.69

Page 118

1.623 1.636 1.62 1.69, 1.69.
4.153 4.230

1.87 Ethene?

		$\bar{X}$	log(x)
Methane	C_1	.57	-.244
Ethane	C_2	1.35	.1303
Propane	C_3	4.19	.622
Blane	C_4	9.21	.964

11.9479 @ Carbon No.

Exponential for guide well. $t = 0.210^{e}$ $r^2 = .995$

or

$\log_{10}(t) = 0.412 \text{ Carbon No} - .661$ $r^2 = .995$

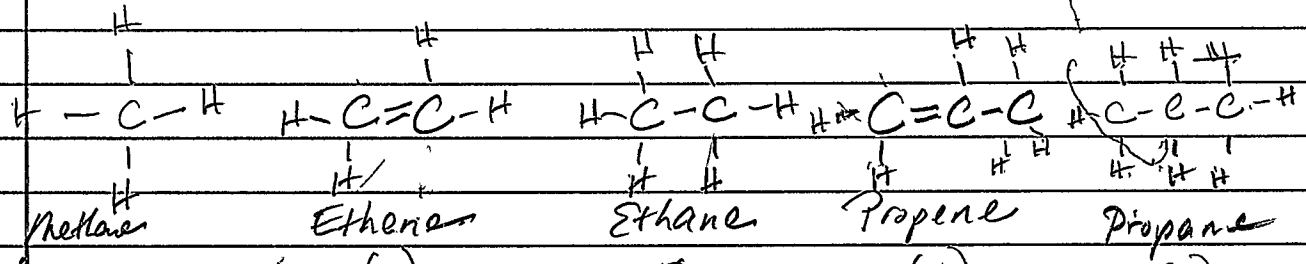
or

$t = 10^{(.412 \text{ Carbon No} - .661)}$

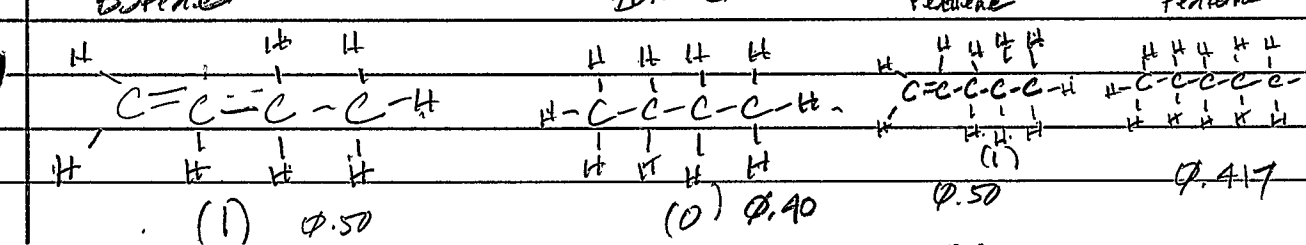
Exponential form for guide well.

$t = 0.210^{e^{0.9479 \cdot \text{Carbon No.}}}$

Unsaturation:
1. Double bonds = +1
2. Ring = +1
3. Triple Bonds = +2



	Methane	Ethene	Ethane	Propene	Propane
Saturation	0	(1) (n ≈ 2.3)?	0	(1)	(0)
CH Ratio	.25	0.50	.33	0.50	.375



	Butane	Butene	Pentene	Pentane
	(1) 0.50	(0) 0.40	(1) 0.50	0.417

Jul 10 2017 Predictive GC Modeling

We have a very good relationship established now for saturated hydrocarbons @ 80°C.

Next question is how is the relationship affected by unsaturated hydrocarbons.

Next question is a source of alkenes. Charcoal powder may be a good source. How is the elution affected by temperature?

The pattern is interesting. All alkenes with a degree of saturation of 1 have a CH ratio of 0.5 Saturated alkanes have a decreasing ratio.

What if we chose log(x) : CH Ratio?

	Predicted from model	CH Ratio	Product	
Methane	(0.56)	.25	(.14)	✓
Ethane	(1.45)	.33	(.48)	✓
Ethene	1.45	.50	0.72	
Propane	(3.74)	.375	(1.40)	✓
Propene	3.74	.50	1.87	
Butane	(9.66)	0.40	(3.86)	✓
Butene	9.66	0.50	4.83	
Pentane	(24.93)	.417	(10.40)	✓
Pentene	24.93	0.50	12.46	

Regression based upon alkane subset.

This is a perfectly linear relationship!

$t \cdot CH_{RATIO} \cong 0.422(t) - 0.146$. $r^2 = 1.00$

OK so...

$$CH.Ratio \cong \frac{0.422(t) - 0.146}{t}$$

Example: Propane: CH Ratio Predicted to be 0.380 vs 0.375 actual

This looks extremely promising.

We also know that if we were to have a $t \cong 0.218$ e 0.9479 - Carbon No. for alkanes only

$$n \cdot \frac{t}{0.218} = e^{0.9479 \cdot CN}$$

$$n \cdot .9479 \cdot CN = \ln\left(\frac{t}{0.218}\right) \quad \text{or} \quad CN \cong 1.055 \ln\left(\frac{t}{.218}\right)$$

Example: Propane: $CN \cong 3.0$ very good;

Two relationships now for alkanes & alkenes:

$$CN \cong 1.055 \ln\left(\frac{t}{0.218}\right)$$ alkanes (alkenes? fractional N?

$$CH\ Ratio \cong \frac{0.422(t) - 0.146}{t}$$ alkanes & alkenes.

Now we apply this to unknowns. Charcoal powder is good test material.

Model assume that it is a hydrocarbon. CO, CO_2 no so O_2, N_2

There are important differences to be had between complete and incomplete combustion processes. They both have their own advantages & disadvantages.

You see now that you can get complete combustion even w/ charcoal powder w/ the simple single outlet/inlet combustion chamber (test tube).

When subjected to incomplete combustion (sealed balloon and/or vacuum evaporation of chamber you reveal the addition of:

1. methane
2. ethane
3. CO

to that of N_2, O_2, CO_2 produced w/ complete combustion

Notice that you are not getting the higher hydrocarbons C_3-C_8 with either method applied to charcoal powder.

Hair piercing higher hydrocarbons more easily

A single hole (syringe) in the balloon will allow some air flow.

Hair in the single puncture balloon collection is performing exceptionally well. Very clean peaks.

alkanes $CN \cong 1.055 \ln\left(\frac{t}{.218}\right)$ *

alkanes alkenes $\frac{1}{CH\ Ratio} \cong \frac{t}{.422t - .146}$

Incomplete combustion produces more subcomponents of the compound. Thus is great for pyrolysis & headspace methods.

Page 120

We have a very clean run on Lair

			MW	Gi	$\frac{1}{CH\ Ratio}$
	.29	O_2	16	.30 (0)	=12.3
	.36	N_2	28	.53 (0)	60.8
	.48	Methane	16	.83 (1)	8.5 (4.2 to 1)
	1.35	Ethane	30	1.92 (2)	3.2 3 to 1, 6 to 2
	1.59	CO_2	44	2.10 (2)	3.0 3 to 1, 6 to 2
C_2 unsat	1.76	Ethene	28	2.29 (?)	2.9 3 to 1, 6 to 2
C_3 sat	4.18	CO (28) or Propane (44) or both	28/44	3.10	2.6 5 to 2, 10 to 4
C_3 unsat	5.11	Propene	42	3.33 (?)	2.5 5 to 2, 10 to 4
C_4 sat	9.55	Butane?	58	3.99 (4)	2.5 5 to 2, 10 to 4
C_4 unsat	12.87	Butene	56	4.27 (?)	2.4 5 to 2, 10 to 4

Notice offset of ~.3 for all unknowns.
These are the alkenes.

	Pentane	72	5
	Hexane	86	6

We see that we can tell quite a lot already to assist in the destruction process with our models.

It is interesting how the H-C ratio is staying relatively constant, however, for both alkenes and alkane (from the model predicting).

My next desire is to look @ Molecular weight.

Ok, we have some revisions of the data for redundancy. Lets recompute the models. Lets use all hydrocarbons in all cases.

	t	CN	CN* Predict	MW	MW* Predict	CN*·MW*	
Methane	.48	1	.66	16	14.8	9.8	2
Ethane	1.35	2	1.65	30	28.2	46.5	
Ethene	1.76	2	1.90	28	31.7	60.2	
Propane	4.18	3	2.72	44	42.9	116.7	
Propene	5.11	3	2.91	42	45.5	132.4	
Butane	9.55	4	3.51	58	53.7	188.5	
Butene	12.55	4	3.77	56	57.2	215.6	

Notice to drop back

Carbon No. – Molecular weight

Good Predictive Model for CN & MW

$CN \cong 0.952 \ln(t) + 1.63$ $\quad r^2 = 0.98$

Quite good actually

Not too bad to have

$CN \cdot H/C\ Ratio \cong 9.83 \ln(t) + 6.98$ $\quad r^2 = 0.87$

Therefore:

$H/C\ Ratio \cong \dfrac{9.83 \ln(t) + 6.98}{.952 \ln(t) + 1.63}$

No good.

$H/C\ Ratio = .091(t) + 3.10$ $\quad r^2 = .30$ Very Poor

H/C Ratio is NOT reliable.

Switch to MW relationship. Very good here:

$MW \cong 13.01 \ln(t) + 24.31$ $\quad r^2 = 0.96$

much better

We have a very good function result here:

$CN^* \cdot MW^* \cong 64.2 \ln(t) + 36.7$ $\quad r^2 = .965$!

great.

Therefore: $MW^* \cong \dfrac{64.2 \ln(t) + 36.7}{0.952 \ln(t) + 1.63}$

Eg, if you had $t = 4.5$ min, you have

$CN^* = 3.06$ Then it is closest to propane, which is correct.

$MW^* = 43.5$

We can also use these models to predict the next component for example

$CN^* = 6.952 \ln(t) + 1.63$

or

$.952 \ln(t) = CN^* - 1.63$

or

$\ln(t) = \frac{CN^* - 1.63}{.952}$ or $t = e^{\frac{CN^* - 1.63}{.952}}$

therefore for $CN^* = 5$ (Pentane or Pentene) $t = 34.5$ min

$MW^* = 642 \ln(34.5) + 36.7 =$

DO NOT USE THIS FOR MW^* Prediction.
It compounds the error.

Use $MW^* = 13.01 \ln(t) + 24.3$

or

$MW^* = 70.4$ gms / mole

What is the molecular wt of pentane? 72.15

Excellent work

Good Predictive Hydrocarbon Models:

Ok, you have a very good model for hydrocarbons now, ie alkanes & alkenes.

You can, given a time of elution

1. Estimate the Carbon number to quite reasonable accuracy by two methods

2. Estimate the molecular weight by two methods

Assume we measure $t = 4.5$ min

$CN \cong .952 \ln(t) + 1.63$ $\quad CN \cong 3.06$

$CN^* \cong 64.2 \ln(t) + 36.7$ $\quad CN \cong 3.04$ } great

$13.01 \ln(t) + 24.3$ $\quad \overline{CN = 3.05}$

and

$MW \cong 13.01 \ln(t) + 24.3$ $\quad MW = 43.9$

$\overline{MW = 43.7}$

$MW^* = 64.2 \ln(t) + 36.7$ $\quad MW = 43.5$

$.952 \ln(t) + 1.63$

This is closest to propane, which is true

Excellent work

and given t, you can reverse estimate CN & MW as well. When reversing the individual regressions will give the best result as they will not combine the errors of each regression. You can try all 4 methods for comparison.

Going forward ie estimate CN* & MW* the combined regression should give you a better result.

The results are too close to be able to distinguish between propane & propene. You can get C_3 w/ a molecular weight, however.

Ok, this is very smooth work.

A very good model for hydrocarbon behavior on the column @ 80°C.

What it does not do is

1. Predict non-hydrocarbon behavior
eg N_2, O_2, CO_2, CO.

What would it do for sucrose pyrolysis, for example, w/ significant oxygen attached?

How would you know?
Like light.

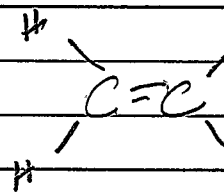
We have a very clean burn of sucrose with peaks of

N_2-O_2
CO_2 (large)
CO (very minor)

You could analyze your CO_2 peak & CO peak as unknowns to see how the model handles them.

Unknown 1 @ 1.563 min
2 @ 4.41 min

(1) CN ≅ 2.06 CN* ≅ 2.17 $\bar{X}$ = 2.11
MW ≅ 30.1 MW* ≅ 31.8 $\bar{X}$ = 31.0



If a hydrocarbon it could only be ethene. But we know that it is not ethene, it is CO_2. Flame test might take care of it. The only problem then would be to determine between ethene and something else close to the C.

MW of CO_2 is 44 t = 1.59 Best non hydrocarbon match.
MW of CO is 28 t = 4.18 Not possible.

So somehow an increase of oxygen is acting like a decrease in molecular weight. I do believe that polarity will be the key here. Is CO_2 polar or non polar?
NON.
CO_2 is polar, therefore it comes out of the column more quickly. CO is polar so it will come out more slowly.

So you can deduce that your best candidate is ethene or another somewhat NON polar compound w/ a higher molecular weight than ethene. Also the carbon number should be between 1 & 2.

Non polarity will reduce the time on the column. Polarity will increase the time on the column, all other factors being equal, which they seldom are.

There is a need to separate between propane & CO. Maybe a different temperature can accomplish this.

Creating a mix of these 2 gases would be a big plus.

We have, however, very good reason to believe that we have both CO_2 & CO being produced by the culture in anaerobic (at least reduced oxygen) conditions.

We know that the cultures are producing both CO_2 & CO under reduced oxygen conditions.

We found papers indicating the production of CO increased by the presence of heme or hemoglobin.

Culture w/ dried blood should now be created & see if CO production is increased.

Culture set up

Set of approx 2 dozen culture w/ test tubes & balloons set up tonight.
~12 w/ sugar, iron & CDB
~12 w/ sugar, iron, CDB, and blood meal
1/2 1/4 2 drops 1/64 tsp

Extracted top layer (protein) from previous set of ~24 cultures into 2 50 ml test tubes marked and dated. Yellow-green color.

Page 130

Journal of Bacteriology
JB Article | Journal Info. | Authors | Reviewers | Permissions | Journals.ASM.org

J Bacteriol. 1972 Dec; 112(3): 1310–1315. PMCID: PMC251565

Carbon Monoxide Production from Heme Compounds by Bacteria

Rolf R. Engel, John M. Matsen, S. Stephen Chapman, and Samuel Schwartz

Department of Pediatrics and Medicine, University of Minnesota Hospitals, Minneapolis, Minnesota 55455

Abstract

Carbon monoxide formation from heme compounds by bacteria was investigated to study microbial hemoprotein catabolism with reference to heme degradation by mammalian tissues. Hemolytic and nonhemolytic bacteria were incubated aerobically and anaerobically with the following substrates: erythrocytes, hemoglobin, myoglobin, cytochrome *c*, hematin, iron hematoporphyrin, copper hematoporphyrin, protoporphyrin, and bilirubin. After 18 hr at 37 C the evolved CO was measured by gas chromatography. None of the bacteria formed CO anaerobically. Under aerobic conditions both alpha-hemolytic *Streptococcus mitis* and hemolytic *Bacillus cereus* formed CO from all of the heme compounds tested, whereas nonhemolytic *Streptococcus mitis* did not evolve CO from any of the substrates. The hemolytic bacteria did not produce CO when the iron of heme was either replaced by copper or removed, as in copper hematoporphyrin and in protoporphyrin, respectively.

Full text

Full text is available as a scanned copy of the original print version. Get a printable copy (PDF file) of the **complete article** (867K), or click on a page image below to browse page by page. Links to PubMed are also available for **Selected References**.


1310


1311


1312


1313

Page 130C

J. Gen. Appl. Microbiol., **31**, 285–292 (1985)

PRODUCTION OF CARBON MONOXIDE BY BACTERIA OF THE GENERA *PROTEUS* AND *MORGANELLA*

ATSUNOBU HAYASHI,[1] HIROSHI TAUCHI, AND SEIICHI HINO[2]

Botanical Institute, Faculty of Science, Hiroshima University, Naka-ku, Hiroshima 730, Japan

(Received July 2, 1985)

A bacterium, isolated from human saliva, produced carbon monoxide (CO) when cultured in nutrient broth containing hemin or hemoglobin. The production of CO was detected by gas chromatography, and confirmed by gas chromatography-mass spectrometry and spectrophotometry which revealed an absorption spectrum of CO-hemoglobin. The isolated bacterium was identified as a strain of *Proteus vulgaris*. Survey of 4 IFO strains of *P. vulgaris*, 1 IFO strain of *P. mirabilis* and 2 IFO strains of *Morganella morganii* (received as *P. morganii*) showed that all the 7 strains had the ability to produce CO. They produced detectable amounts of CO when cultured in a glucose-peptone medium or in the nutrient broth without the addition of heme compound, and the CO production was distinctly enhanced by the addition of hemin. *Morganella morganii* IFO 3168, the most active CO producer among the strains tested, produced about 2 μmol of CO in the absence of hemin and 6 μmol of CO in the presence of 3 μmol of hemin. The result suggests that CO production by the *Morganella* strain is different from the CO production by mammalian tissues where 1 mol of protoheme is degraded to 1 mol each of CO and biliverdin.

Small amounts of CO are produced in normal human tissues where hemoglobin is degraded to equimolar amounts of biliverdin and CO. The degradation is catalyzed by microsomal heme oxygenase (*1*) and the CO is produced by the oxidation of α-methene bridge carbon of the porphyrin ring (*2*). Some reports showed that abnormally large amounts of CO were produced in some tissues of post-mortem human bodies (*3*, *4*). The cause of the abnormal formation is unknown and we suspected that bacterial activity might be responsible for the formation. ENGEL et al. observed CO formation by hemolytic strains of *Bacillus cereus*

[1] On leave from the Scientific Investigation Research Laboratory, Hiroshima Prefectural Police Headquarters, Hiroshima, Japan.

[2] To whom correspondence should be addressed.

Reduced O_2 culture (most likely actually anaerobic) extracted top layer does indeed pass the Bradford test w/ $\lambda_{max} = 621$ nm.

We do have significant generated protein directly from the culture (reduced O_2).

Incubation period in the case is ~ 90 days.

We have made good progress with the GC the last week. We are quite comfortable w/ C1 - C4 hydrocarbons now (alkane and alkene a projection into C5 is no problem.

O_2, N_2, CO_2 & CO are all treated separately for now.

Altogether, we are covering

O_2	Propane
N_2	Propene
CO_2	Butane
CO	Butene
Methane	Pentane (projected)
Ethane	Pentene (projected)
Ethene	

along w/ developed pyrolysis techniques.

Regroup on Projects

1. Study w/ [redacted] this week.
2. Citizen samples
3. Simulate tryptophan, glutamic acid & proline in IR? spec?
4. Production of DNA
5. ICM release
6. UV software purchased?
7. GAMESS work continued
8. Davis Courses
9. Combustion analysis, CH ratio
10. Revisit, the MW of the secreted protein would be helpful
11. Month cultures
12. Microwave digestion
13. Mark Ball pyrolysis?

Triglycerides (fish oil) should work as borderline sample in GC.

Jul 12 2017 Page 133

[redacted] Study

1. Microscopy
   - Low power
   - Mid Level
   - Higher Power
2. Urine Analysis
3. Blood –
4. Tissue Sample
5. Wet mount

1. Wire Test
2. Vit C Culture →

[redacted] study included:

1. Macro photographs taken of leg.
2. Lower power USB microphotographs 20-600x on your skin
   Observed skin cells and attempted to establish a low power reference
   Concentration locations (green)
   Filaments (only 2)
   Fluid
3. Eject Extracted deposits - Crystals under low USB.
   Too thick before observation beyond surface structure. Green color is reinterpreted dust mite material for solubility test
4. Skin scrapings
   1. Skin cells also observed as reference pt under low power
   2. Spherical show up @ 1500x & hints of filament network @ 1500x
   3. 3000x - Filament network is verified (Scope may be under less than ideal conditions) & spherical structure separated from air bubble possibilities

5. at the end of the section, I have an high of coincidence between the culture fluids that have been developed and manifestation in a skin condition, but only identifiable @ the level under high power magnification @ ~5000x

The especially involves around the filament networks, the spheroid structure & the blue traits within.

6. We then go to urine testing [redacted] shown appearance of presumably large amounts relatively of lymphatic fluids that have effectively corralled large numbers of CDB. These are remarkable photographs that demonstrate highly efficient activity w/in the particular immune system. Health practitioners must become aware of the response by the body when these now known to be possible.

It is fascinating that the last lymphatic testing is occurring simultaneously w/ a significant skin manifestation that has appeared only over the last 6 weeks

7. Blood tests also conducted @ 5000 X.
Two samples. Both samples come out
especially clean & intact - first appear
quite favorable relative to past studies.

Jul 13 2017

1. Culture photos
2. Citizen sample
3. Forward drug
[redacted]
6. Melting point
7. Software set up
8. IR
9. Ultrasound

Extricate UV analyser: (We have preliminary IR analyser available.

Colby database 228 nm
277 nm

There are numerous candidate with
1. 228 & 277 nm
2. Carbonyl present
3. Saturated & unsaturated CH

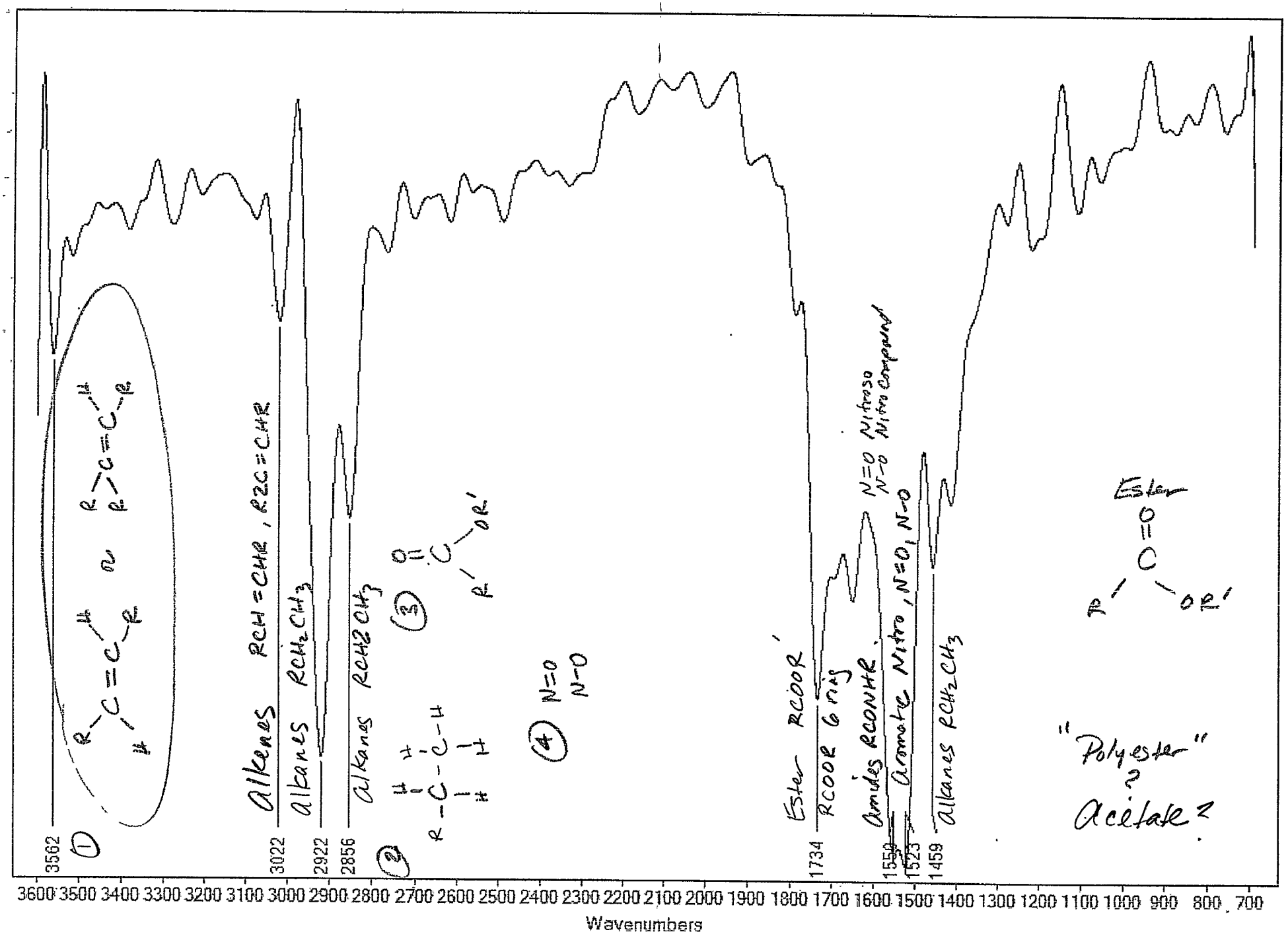
Phenethyl Acetate is one Candidate of interest.

There a Case where GAMESS simulation of IR spectra could be useful.

Exfoliate IR Analysis - Preliminary

Limited Sample Material Available

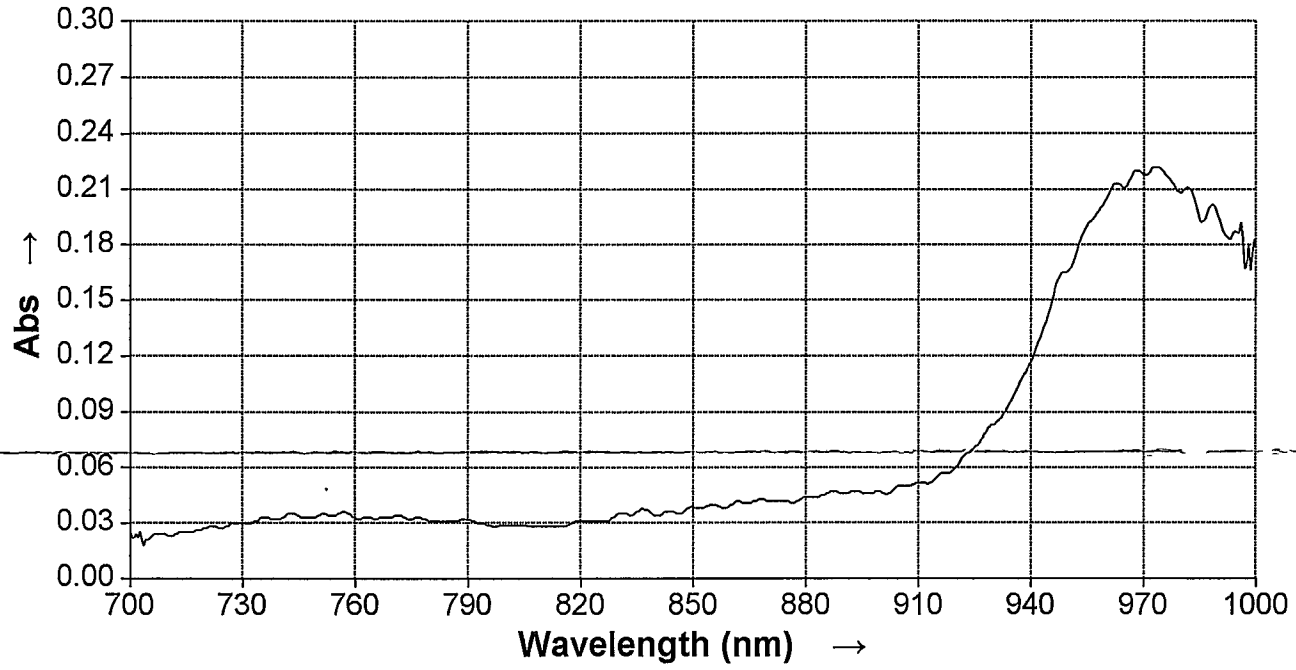
Morgellons Skin Efoliation [redacted] Jul 12 2017 ATR Methanol Film Layer - 01.JPEG



SPECTRONIC 200

Scan report

Spectrum of :	Distilled	Analysis date :	02 - Nov - 2015
Analyzed by :	CI	Analysis time :	4:09:43 PM
Channel # :	0	Print date :	02 - Nov - 2015
		Print time :	4:13:05 PM



The strategy being used right now is the following.

1. Identify a candidate compound & molecular formula for it.

2. Model the compound w/in Avogadro and submit it to GAMESS computational package w/ IR output.

3. Scale the IR output appropriately & view it in comparison to actual IR plot.

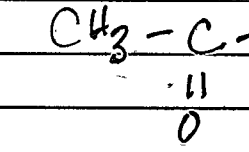
4. Adjust the molecular proposal and attempt until you have reasonable convergence.

As an example, the exfoliate IR spectra match w/ an existing compound can be pursued with:

NIST best match appears to be
2 Chloro para acetotoluidide

SDBS Formamidine acetate
Eq $CH_4N_2C_2H_4O_2$

Seems to me that Ethyl acetate is in the right general category.



Ethyl acetate is soluble in water. $C_4H_8O_2$
It is a liquid.

Aluminum melts @ 660°C.

—

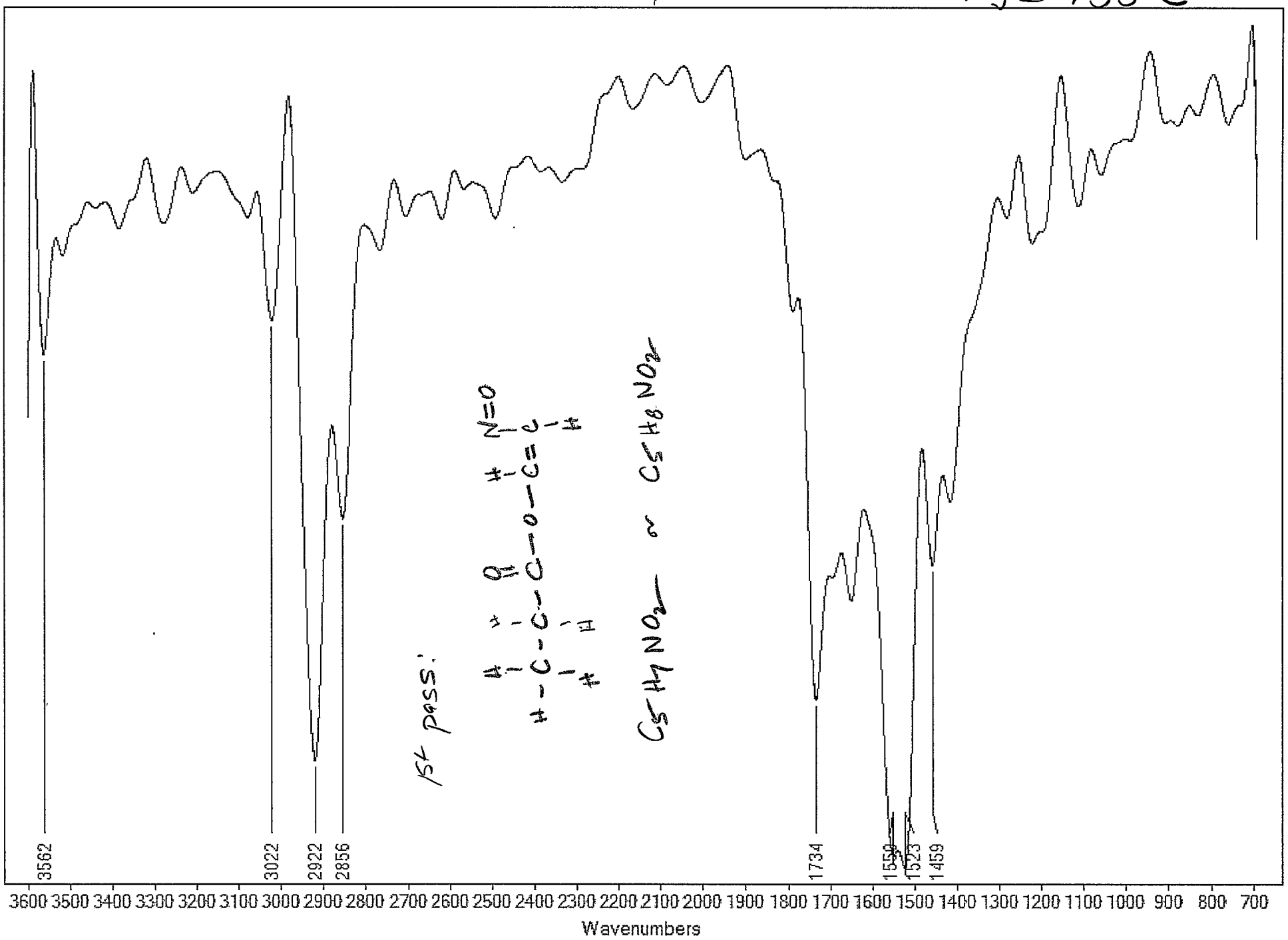
We see that the explorate never melts. It begins charring @ ~ 240°C. Would love to distinguish between them.

—

We can heat up the melting block to 250°C by timing the rate of heat increase.

Morgellons Skin Efoliation [redacted] Jul 12 2017 ATR Methanol Film Layer - 01.JPEG

Page 138 C



Digi-Sense melts goes to 1200°C!

t	temp°C	t	temp	t	temp
0	65				
1	72				
2	80				
3	86				
4	91				
~~5~~	~~94~~				
6	98				
7	105				
8	113				
9	125				
10	145				
11	168				
~~12~~	~~181~~				
14	206				
18	241				
11	~~90~~ 284				
13	311				
16	341				
17	362				

The digital thermistor is highly valuable. Now I can now get up to 450°C for melting point work. The range great.

It is close to linear $r^2 = .908$

But logistic is even better

Logistic is a very good model.

Today I have extracted the larger scale ("LAN1") culture the top layer which we know to contain a protein. Past work indicate about 70% of the volume is water. The layer is not red @ the time, it is somewhat olive colored.

It would be good to concentrate the protein, although heat may well denature it.

These notes for LAN1 occurred on Jun 16 2017.

250 ml H_2O
1 tbsp sucrose
1 tsp $FeSO_4$
1/8 tsp salt (NaCl
CRB

I will now start 4 of these cultures. It was called large scale anaerobic culture but actually it is only reduced oxygen & primarily incubation.

We see now that we have allowed LAN1 to incubate @ ~ 1 month @ ~ 90°F S. It should be called Large scale Reduced Oxygen series, or LRO, n.t CAN. Make 4 cultures.

These 4 cultures have been set up:

LRO 1
LRO 2
LRO 3
LRO 4

I have also established an evaporation glass pan to remove water from the extracted protein in an effort to concentrate it.

It is also noted on me that a candidate in the 1st HPLC separation was Ethyl acetate, at least as a candidate for further examination.

It is now noted that one of my classes of materials under examination for skin exfoliation under Morgellons symptoms is also Ethyl acetate!

Double coincidence, must she deserve further examination & comparison

Jul 15 2017

The Bradford reagent control sets in again, when tested @ 630 nm. The shift is critical (but small) to indicate the presence of protein at $\lambda = 619-623$ nm.

I attempted to evaporate the extracted layer from the reduced oxygen cultures. It sat too long due to company so the proteins have been charred. Start again - approx 1 month of incubation time is required.

I can use the charred material for GC-pyrolyser analyses. I have also returbed some to solution although it is now dark brown. It may be useful for some intermediate tests.

The charred material in solution no longer passes the Bradford test. It is no longer a protein form so it is unlikely to be useful. Pyrolyser analyses is still ok.

The GC examination @ this point of the charred material is that we will be dealing w/ CO_2 & H_2O products of combustion. There are no sequential hydrocarbons coming into play - it truly may be just to test that we have left them.

The good news is that we have another supply of the secreted protein held in reserve that will be adequate for testing until cultures produce additional protein.

The device material does pass the Bradford test w a shift from 630 to 625nm.
It is adequate for detection.

Now what is strange is that both tungsten & UV lights were turned off, so how did it get any reading?

We have recalibrated reagents and repeated the Bradford test. Now we have a λ max of 627 nm. There is not a strong but we do still have a shift indicated. It means that our protein concentration in the sample might be rather low.

We see now that the GC analysis of the charred protein is hardly devoid of activity. At 21 & 25 min w/ a ramp up to 220°C we have 2 peaks. These need to be investigated.

I take the device result back.

There is tremendous activity here @ 220°C.
I have a half dozen peaks w/in 3 min.
You will need to do some calibration @ 220°C.

Your method for polymerization.
Heat the balloon in a press sense & then vacuum evacuate the balloon.

Then you reheat until pressure is again sufficient for partial inflation and take off samples @ that time.

We have some real activity here @ 220°C w/ the Charred Reduced O_2 protein. We need to recalibrate & repeat a phase in Calibration @ 150°C.

Baking the column @ 150°C seems to be bringing out a great deal of new material. There must be 20 items coming out of the column @ 150°C.

We already have some information about how time affects the column:

	Actual	Predict	$\Delta t = 70°$	
Peak	100°	100°	150°	220°
		0.35	0.35	0.36
		.86	0.65	0.46
		2.40	1.27	0.84
		3.80	1.84	1.13
		6.20	2.81	1.44
		11.63	5.01	2.77
		30.92	12.82	5.60

(margin note: you do not know what anything here is, you are determining only relationship. This temp is fine! We lose these @ 100°C)

(margin note: This model stays valid)

This change is linear: $t_{220} = 0.422\, t_{150} + 0.306$

or $t_{150} = 2.349\, t_{220} - 0.692$ $r^2 = .992$

$t_{220} - .306 = 0.422\, t_{150} \Rightarrow \frac{t_{220} - .306}{t_{150}} = 0.422$

We know that $0.306 \cong O_2(t)$

so that $\frac{t_{220} - t(O_2)}{t_{150}} \cong 0.422$

$\frac{220°}{150°} = 1.47$

$\frac{150°}{220} = 0.68$

Now, notice that $\ln\left(\frac{220}{150}\right) \cong 0.383$

This is not too far from 0.422

Could we have:

$$\frac{t_{220} - t(O_2)}{t_{150}} \overset{?}{\cong} \ln\left(\frac{220^\circ}{150^\circ}\right)$$

Not Good Enough

Lets apply to 100°C and see how it does.

??? $\quad t_{220} \cong t_{150} \cdot \ln\left(\frac{220}{150}\right) + t(O_2)$

This would infer that

? $\quad t_{100} = \frac{t_{150} - t(O_2)}{\ln\left(\frac{150}{100}\right)} = 2.47\left(t_{150} - t(O_2)\right)$

Both to be only approximate but still helpful to some degree. What we see is that

60-80° low temps excel in the lower hydrocarbons
150° mid temps are a good compromise of investigation.
220° excels w/ the higher hydrocarbons and may well miss the lower HC's, exactly the reverse of case 1 for low temps.

We can see that the best serial results were achieved @ T = 150°C.

More work, but also gain some.
Recommend that we shift to this mode for general investigation.
You'll see a lot more w/ it.

Now we will Calibrate the 80° model to 150°
Let's use propane & butane as our standards.

We have a good model @ 80°C
We know the relationship between 150 & 220
Now Calibrate my 80° model to 150° and
then we will be able to extend that to 220°C.

You can also put out the 220's on the end
as a catch all feature. ie
150° for 20 min)
Ramp) to 220
Hold) for another 10 min.

You are keeping most of the water out of the column
which is great. Building a styrofoam cup
would be a good project.

We have our answer for propane @ 150°C. t = 0.98
@ 80°C t = 3.38 min (trials also)
3.52
X = 3.45 3.46

Now for butane:

Butane @ 150°C : t = 2.20.

You are getting very clean peaks @ 150°C which you now know and expected. Mid range compromise is best.

Gas	80°C	150°C	220°C
O_2	0.29		
N_2	0.36	0.36	0.36
Ethane	1.35	0.67	0.46
CO_2	4.18	1.26	0.84
Propane		2.02	1.13
Butane	9.3?	2.81	1.48
Pentane		5.01	2.80
Hexane		12.82	5.67
Methane	0.48		
Ethene	1.76		
Propene	5.11		
Butene	12.55		
			13.56

Carbon Number Prediction
Molecular Weight Prediction
Time Conversion Relationships for GC

Important Page

Page 150

$t_{150} \cong 2.34\, t_{220} - 0.65$ $\quad r^2 = .992$

$t_{220} \cong 0.424\, t_{150} + 0.29$ $\quad r^2 = 0.992$

$t_{80} \cong 5.44\, t_{150} - 2.06$ $\quad r^2 = 0.984$

$t_{150} \cong 0.181\, t_{80} + 0.39$ $\quad r^2 = 0.984$

FOR HYDROCARBONS (Not Polar Molecules)

We also have our predictive models for CN & MW @ $T = 80^\circ C$

$CN \cong 0.952 \ln(t_{80}) + 1.63$

$CN^* \cong 64.2 \ln(t_{80}) + 36.7$ / $13.01 \ln(t_{80}) + 24.3$ — not necessary

and

$MW \cong 13.01 \ln(t_{80}) + 24.3$

$MW^* \cong 64.2 \ln(t_{80}) + 36.7$ / $0.952 \ln(t_{80}) + 1.63$ — Not necessary

These can be recreated for a base of 150°C or extrapolated from the 150–80 relationships w/ increased error, your choice. For now, extrapolate.

For 150°C:

$CN_{150} \cong 0.952 \ln(5.44 t_{150} - 2.06) + 1.63$

$CN^*_{150} \cong 64.2 \ln(5.44 t_{150} - 2.06) + 36.7$ / $13.01 \ln(5.44 t_{150} - 2.06) + 24.3$ — not necessary

$MW \cong 13.01 \ln(5.44 t_{150} - 2.06) + 24.3$ — not necessary

$MW^* \cong 64.2 \ln(5.44 t_{150} - 2.06) + 36.7$ / $0.952 \ln(5.44 t_{150} - 2.06) + 1.63$

We can now apply these models to the charred protein.)

We have, within the charred protein (180°C), already identified:

Ethane C_2
Propane C_3
Butane C_4
} sum = C_7

Now we have additional peaks @ t$_{1/2}$ of
2.81 min
5.01 min
12.02 min

Therefore:

t	CN	CN*	$\overline{CN}$	MW	MW*	$\overline{MW}$
2.81	4.1	3.5	$\frac{3.8}{2}$ = 4.0	51.9	49.5	53.1 = 54

Therefore the model predicts a CN of 4 and a MW of 54

Since we have already positively identified butane we know that the t = 2.81 min @ 150 = butene
Very good work. MW was also closest to butene as well.

We now have Ethane C_2
Propane C_3
Butane C_4
Butene C_4
} sum = C_{13} min

We now have t= 5.01 min @ T=150°C. Using models

t	CN	CN*	$\overline{CN}$	MW	MW*	$\overline{MW}$
5.01m	4.1	3.1		66	52	

Clearly the simpler first model is closer and sufficient.

We can see that we are dealing w/ pentane here = C_5
Pentane is C_5H_{12} w/ a MW of 72

We now have Ethane C_2 Sum = C_{18}
Propane C_3
Butane C_4
Butene C_4
Pentane C_5

Next t	CN	CN Predicted	MW	MW Predicted
12.82	5.64	C_6 C_5	79.1	10 72 or 86

We are closer to hexane in both cases. The model predicts hexane @ C_6H_{14}

This means we have: Ethane C_2
Propane C_3 Sum = C_{25}
Butane C_4
Butene C_4
Pentane C_5
Hexane C_6

For a saturated hydrocarbon we expect a maximum of $C_{25}H_{52}$
MW max = 352 (but we have ~ 4000 already)

Reduced Oxygen Protein Analyzer by GC

Page 15

Thx for very good work on the column.
We have now proceeded through hexane
in very good order.

Are there any additional peaks @ 220°C?

We will need to bring in CO & CO_2 into
the picture next so we can almost certainly
predict their appearance.

We know that
$t_{150} \cong 0.181 t_{60} + 0.39$

Prediction of CO_2 & CO @ 150°C

We know
CO_2 @ $t_{60} = 1.59$
CO @ $t_{60} = 4.18$

Therefore
$t_{150} \cong 0.68$
$t_{150} \cong 1.15$

We therefore see that CO_2 overlaps
and is indistinguishably from ethane in this regard.
CO_2 comes out slightly later than ethane.
You therefore expect a trailing peak @ ethane
and guess what, you have one.

You can separate ethane & CO_2 @ 80°C
but not @ 150°C. Thx shown you the
value of your predictive model and
of varying the temperature of the column
when substances are in competition
w/ one another.

Thx an excellent work on the column.

Very strong hexane peak showing up @ 150°C w/ charred protein. You may also have some additional unknowns showing up now.

CO_2 predicted @ $t = 0.68m$ @ $T = 150°C$

CO predicted @ $t = 1.15m$ @ $T = 150°C$

We know that we have removed almost all H_2O from the protein via charring & the GC results confirm that.

We do see additional activity with the ramp up to 220°C. It appears that we have 2 more peaks. This is likely to bring in up to C_7 and/or C_8. This brings the compound to C_{40}. This brings a saturated HC to $C_{40}H_{82}$. This brings MW to 532 by GC alone. There is good work and probably quite difficult normally to achieve w/ GC.

What is the molecular formula of the smallest protein known?

It has been unexpectedly beneficial to have created the charred version of the protein. It is perfectly suited to pyrolysis analysis.

Jul 16 2017

I would like to see how CO_2 gets in the model since it is non polar.

Let's analyze the Chromatogram @ 220°C ← Charcoal Pistol.

First pick @ Ø.49

We know

$t_{80} \cong 5.44 t_{150} - 2.06$

$t_{150} \cong 2.34 t_{220} - .65$ Not adequate

Therefore

$t_{80} \cong 5.44(2.34 t_{220} - .65) - 2.06$

$\cong 12.73 t_{220} - 3.536 - 2.06$

$t_{80} \cong 12.73 t_{220} - 5.60$

and

$CN \cong Ø.952 \ln(t_{80}) + 1.63$ OK

$MW \cong 13.01 \ln(t_{80}) + 24.3$

for $t_{220} \cong Ø.49$, $t_{80} \cong Ø.64$ Not adequate

$CN \cong 2.2$ Closest is Ethane $CN = 2$

$MW \cong 32$ $MW = 30$

Very good.

Page 156

$t_{220} \cong .95.85 \Rightarrow t_{80} \cong 6.49$ 6.28. This is wrong.
$CN \cong$ "
$MW \cong$ t_{80} should be ~ 4.18

We need a new relationship established for $t_{80} \rightarrow t_{220}$

	80°C	220°C	
N_2	.36	.36	
Ethane	1.35	.46	OK
Propane	4.18	.84	
Butane	9.3	1.13	

$t_{220} \cong .087 t_{80} + 0.37 \quad r^2 \cong .96$

$t_{80} \cong 11.056 t_{220} - 3.91 \quad r^2 \cong .96$

5. try again.

$CN \cong .952 \ln(11.056 t_{220} - 3.91) + 1.63$

$MW \cong 13.01 \ln(11.056 t_{220} - 3.91) + 24.3$

for $t = 0.49$ $\hat{CN} = 2.0$ vs $CN = 2$ Ethane,
$\hat{MW} = 29.6$ $MW = 30$ Positive ID

for $t_{220} = .85$ $\hat{CN} = 3.2$ Ethane/CO? $CN = 3$ ~~Propane?~~
$t_{80} \cong 5.49$ $\hat{MW} = 46$ $MW = 44$ ~~Positive ID~~

for $t = 1.06$ $\hat{CN} = 3.5$?? Propane: $CN = 3$ Butane: ~~$CN = 4$~~
$\hat{MW} = 51$ This is Propane $MW = 44$ " ~~$MW = 58$~~
There is some uncertainty here.

for $t = 1.52$ $\hat{CN} = 4.0$ This is positive ID for Butane
$\hat{MW} = 58$ $CN = 4 \cong MW = 58$

for $t = 2.03$ $\hat{CN} = 4.4$ This could be Butene
$\hat{MW} = 62$ most likely

for $t = 2.82$ $\hat{CN} = 4.8$ vs $CN = 5$ Pentane
$\hat{MW} = 67$ $MW = 72$

Good work falling into place here

for $t = 5.14$ $\hat{CN} = 5.5$ ~~Hexane~~ ~~$CN = 6$~~ This must be pentene
$\hat{MW} = 71.5$ ~~$MW = 86$~~
~~This is hexane. Should about 10.~~

for $t = 13.56$ $\hat{CN} = 6.4$ This is hexane. $CN = 6$.
$\hat{MW} = 89$ $MW = 86$

We have made good progress. There are still some peaks that are unclear.

80 150 220

O_2
N_2
Methane
Methene
Ethane
Ethene
CO_2
Propane
Propene

also

$$\hat{MW} = 16.26 \ln(0.47 t_{150} + 0.17) + 45.9$$

$$CN = 1.18 \ln(0.47 t_{150} + 0.17) + 3.16$$

and

$$\hat{MW} = 16.26 \ln(.091 t_{80} + 0.44) + 45.9$$

$$CN = 1.18 \ln(.091 t_{80} + 0.44) + 3.16$$

Page 158

CN		80 CN*	MW*	150 CN*	MW*	220	MW
	O_2						
	N_2	.36		.36		.36	~30
1	Methane	.48					
	Methene						
2	Ethane	1.35	2.37	0.67		.47	30
	Ethene	1.69					
	CO_2	4.18		0.73			40
3	Propane	4.19	2.4 43	1.27		1.10	44
	Propene	5.11		1.90			42
4	Butane	9.3		2.40	3.6 52.4	1.50	58
	Butene	12.55		4.25		2.03	56
5	Pentane			5.4	4.4 63.3	2.82	72
	Pentene			13.2		5.74	70
6	Hexane					13.56	86

$t_{80} \cong 5.32\, t_{150} - 2.30$ $\quad r^2 = 0.97$

$t_{150} \cong 0.17\, t_{80} + 0.42$ $\quad r^2 = 0.97$

$t_{150} \cong 2.10\, t_{220} - 0.35$ $\quad r^2 = 1.00$

✓ $t_{220} \cong 0.47\, t_{150} + 0.17$ $\quad r^2 = 1.00$

$t_{80} \cong 9.64\, t_{220} - 3.65$ $\quad r^2 = 0.88$

✓ $t_{220} \cong 0.091\, t_{80} + 0.44$ $\quad r^2 = 0.88$

$MW \cong 16.26 \ln(t_{220}) + 45.9$ $\quad r^2 = 0.92$

$CN \cong 1.18 \ln(t_{220}) + 3.16$ $\quad r^2 = 0.93$ good

Current models for hydrocarbons or non-polars.

also:

$MW \cong 16.26 \ln(0.47\, t_{150} + 0.17) + 45.9$

$CN \cong 1.18 \ln(0.47\, t_{150} + 0.17) + 3.16$

MW = Molecular Weight
CN = Carbon Number

Current GC Models

(for hydrocarbons & non polars)

150°C:

$MW \cong 16.26 \ln(\emptyset.47 t_{150} + \emptyset.17) + 45.9$

$CN \cong 1.18 \ln(\emptyset.47 t_{150} + \emptyset.17) + 3.16$

220°C:

$MW \cong 16.26 \ln(t_{220}) + 45.9$

$CN \cong 1.18 \ln(t_{220}) + 3.16$

80°C:

$MW \cong 16.26 \ln(.091 t_{80} + \emptyset.44) + 45.9$

$CN \cong 1.18 \ln(.091 t_{80} + \emptyset.44) + 3.16$

and time relationships are:

$t_{80} \cong 3.36 t_{150} - 1.13$

$t_{150} \cong \emptyset.28 t_{80} + \emptyset.41$

$t_{150} \cong 2.14 t_{220} - \emptyset.37$

$t_{220} \cong \emptyset.47 t_{150} + \emptyset.17$

$t_{80} \cong 9.64 t_{220} - 3.65$

$t_{220} \cong \emptyset.091 t_{80} + \emptyset.44$

These are valuable predictors that allow accuracy in column temperature selection.

They also assist in HC & non polar component identification independent of column temperature.

CO_2, CO, polar molecules would be another topic to bring into the modeling. CO_2 is non polar. O_2, N_2 also non polar.
These allow for some rough estimates w/ the molecular weight portion of the model.

Test the 150°C column w/ CO_2.

Baking Soda & Vinegar Ø.733
Hold Breath Ø.730

The fact that CO_2 behaves radically different than hydrocarbons on a function of temperature on the column is very beneficial.
This can be used to separate CO_2 from the hydrocarbons

Next you need to identify CO @ 150°C also.
We also need to distinguish Ethane & CO_2 @ 150°C if possible (0.65m)
On 150°C Ethane appears to come out slightly before CO_2 (Ø.73m)

The Control file is the one they for temperature control & duration of run.

The Components are not saved in the Control file. They are their own file to edit and save & LOAD within the Edit Channels window.

We can see that the Channel Protein is composed of ethane (majority) from 150°C
propane
propene
butane
butene
pentane from 320°C
pentene
hexane

C2
C3
C3:
C4
C4:
C5
C5:
C6

$E = C_{32}$ If saturated this would be $C_{32}H_{66}$

minimum

How can you get CO_2?

We have now proven today that the CDB reduced culture is producing hydrocarbons We now have a record of

CO_2
CO
propane
butane
(likely ethane)

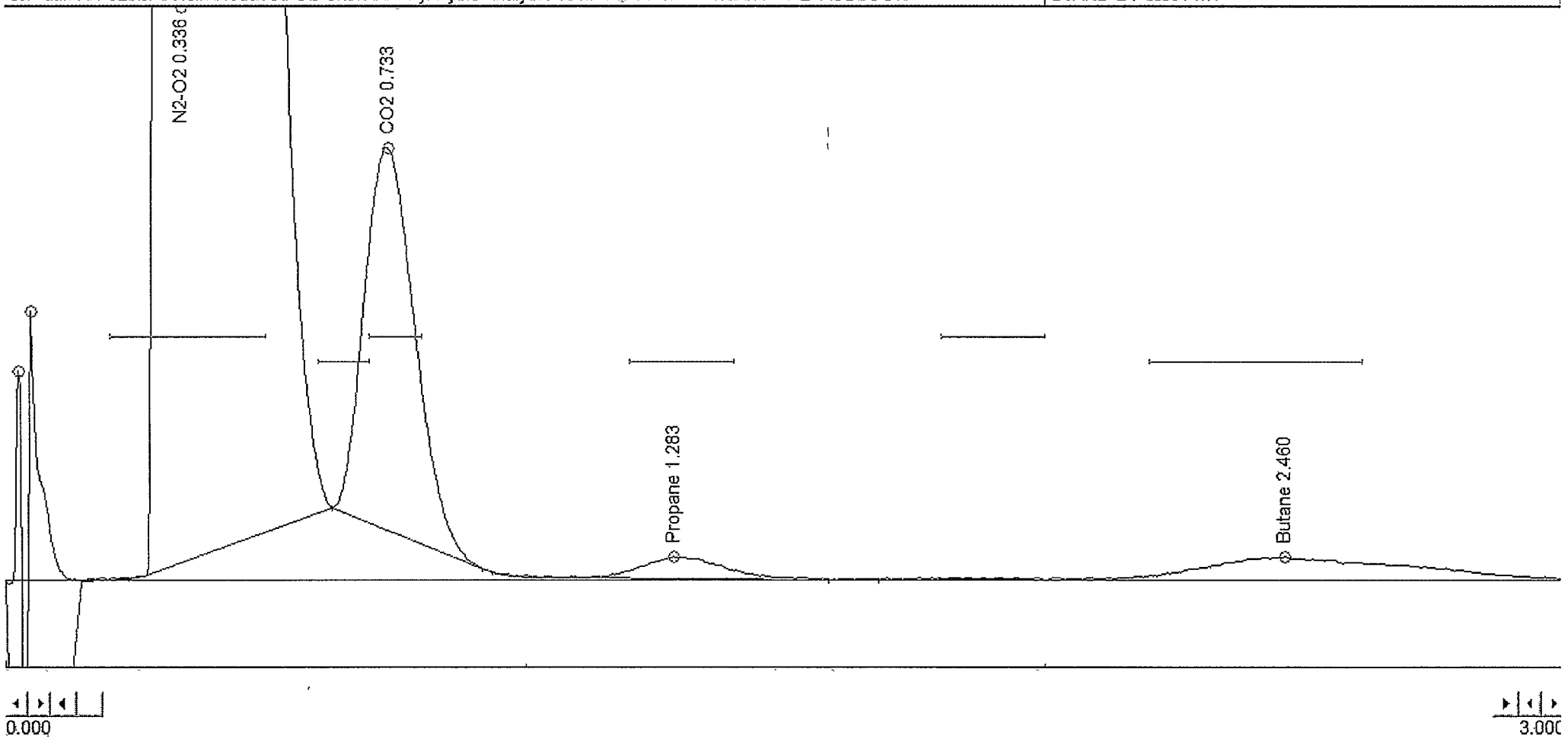
gas production by the specific variation on CDB growth.

CDB Hydrocarbon Gas Production Verification

Protein formed known to be a min of C32

Molecular Weight Estimate in Protein is Currently ~4000 Da

CDB Reduced 02 Culture - Hydrocarbons Produced 150D Jul 16 2017



In 2014, it was quite the news that bacteria had been genetically engineered to produce propane.

We have propane & butane directly from the culture. Genetic engineering anyone?

We have the protein directly from the culture. Genetic engineering anyone?

With 150°C we have two new peaks showing up w/ charcoal.

One is about 1.15 & the other about 13.2

MW = 76

CN = 5.3

We have some additional peaks that have come from the charred protein @ 150°

Should be pentene

CN = 5

MW = 70

1.27 area is propane but we have a double peak @ 1.23 & 1.27

HC Model GC

We now have some new data:

CN		80°	150°	220°	MW
0	O_2	.29			32
0	N_2	.36	.36	.36	30
1	Methane	.48			16
1	Methene				14
2	Ethane	1.35	.67	.47	30
2	Ethene	1.69			28
	CO_2	4.18			40
3	Propane	4.19	1.27	.410	44
3	Propene	5.11			42
4	Butane	9.3	2.40	1.50	58
4	Butene	12.55	4.25	2.03	56
5	Pentane		5.4	2.82	72
5	Pentene		13.2	~~2.9~~ 5.74	70
6	Hexane	~~9905~~ predicted	27.4 predicted	~~13.56~~	86

$MW \cong -13.84 \ln(t_{150}) + 40.2$

$CN \cong 1.04 \ln(t_{150}) + 2.7$

Current Model

$t_{150} \cong 0.30\, t_{80} + 0.1$ $\quad r^2 = 0.94$

$t_{80} \cong 3.10\, t_{150} + 0.2$ $\quad r^2 = 0.94$

Looks pretty good.

$t_{150} = 2.44\, t_{220} - 1.0$ $\quad r^2 = .99$

$t_{220} = 0.40\, t_{150} + 0.4$ $\quad r^2 = .99$

$t_{220} \cong 0.128\, t_{80} + 0.4$ $\quad r^2 = 0.96$

$t_{80} \cong 7.50\, t_{220} - 2.7$ $\quad r^2 = 0.96$

Now lets start looking @ solvents, preferably less polar.

Start w/ MEK - C_4H_8O Xylene C_8H_{10}

The polarity index is 4.7
Water is 9.0
Xylene is 2.5
Acetone is 5.1
Methanol is 5.1
Hexane is 0.0
Toulene is 2.4

So MEK is still fairly polar.
We probably need to try xylene.

Xylene will be a little hard to push through the column.

Maybe the peak I saw was MEK.
Nothing is coming through the column @ 220°C.
The column looks clean.

We may have a very clean peak of MEK @ 1.16 min @ 150°C.
Lets back off to 80°C

We have a problem, the gas is not flowing
For some reason, the gas flow a pressure has
decreased and you have lost all the calibration
that you have done. For some reason the flow rate
must now be set much higher and I do not know
why. Everything is later now coming at the column.
And I am not sure MEK is even coming out.
I do however, now have a very clean baseline.
My flow rate was at 6, now it is @ 15.
No idea whatsoever why I had to do this.

CO_2 is now 2.126 but it was 1.59
O_2 is @ 0.41 it was 0.36
Ok, the ratios look constant. It is now 1.33
same longer than it used to be. We should be able to
adjust for this, even w/ our existing models.

I am not sure why the flow rate change w/ the
gas, however.

You have boosted the flow rate from 6 to 20.
The instrument now looks proper and as it should
proper but I have no reason why the gas flow
dropped so sharply. Everything @ this point look
fine except for the unexplained change.
The gas flow almost stopped .. your test tube
was a life saver here.

I did not have luck w/ the solvent MEK trial
today.

I have now boosted it to 22.
It is interesting that the column says silica gel

Then a bazaar. I have the silica gel column installed instead of the Hayes Sep D!
How did that happen!?

You generally see a peak coming out @ 220°C @ 15 min!

You have the wrong column installed!

The gas flow might have opened up again after baking the septa and the MEK peak is now coming out. It looks really fast.

Something was plugging up the column here.

Now we have a huge peak coming out @ 220°C @ 17 min.

I have decreased flow rate down to 12 now and it looks pretty decent.
Not sure, you need 22.

Something is happening @ the injector port.
Now it is going fast again.

It is ready to go to B now.

Clearly something has happened @ the injector port.
Consider acetone

The problem was that the septum was beat up!
I have fixed it and have flow rate back to 6!

With charcoal, Hayes Sep D has given me
9 separate peaks @ 150°C w/in ~ 4 min.
The looks very good. You have flow rate @ 12.
You may need to lower it some back to 8 or 10.
11 w/in 1 min.

Why did you not have the column installed?

Jul 17 2017

I have an oxygen meter now: 0-100%
This is going to be very valuable.
We captured gas in a larger balloon.
One needle goes to GC
One needle goes to O2 meter.
Our first test reading decreases O2 from 19.0%
to 10.8%.
This is a 8.2% reduction w.r.t. to surrounding air.
and a 12.7 39.2%
20.9 19.0 - 10.8 = 43.1% reduction w/ respect to
20.9 19.0 available oxygen.
20.9 → 17.4% 16.7%
18.6 → 15.2 = 18.3% Reduction
in oxygen relative to
available O2
So if O2 is 19.0%, then = 3.5% reduction

Example, area of N2O peak is 2358 units.

If O2 = 19% & N2 = 81% @ 2730' elevation
then normally
N2 area = 1910.0
O2 area = 448.0
but actual O2 area is 19% - 3.5% = 15.5%
.155 (2358) = 365.5 area
.845 (2358) = 1992.5 area

There is only one adjustment we have to remember to make @ 20.9%
This is wrong
O2 @ any altitude is still 21%.

Theyse $\frac{365.5}{2358} = 15.5\%\ O_2$

$\frac{1992.5}{2358} = 84.5\%\ N_2$

and the are how the peak splits up.

Let see if it possible to develop the CH ratio from these data.

Using the Hayes Sep D.

We have a marvelous & clean MEK solvent peak in short time @ 150°C. This IS great! Also a very small & clustered air peak which is also helpful as a reference. The column shows great promise.

Ther mean there is that dissolve in MEK should be useful. E.g. Can we add an aromatic oil to the test?

The chemical formula for styrofoam is C_8H_8. It appears to dissolve completely within MEK. Is it ok to place this polymer in a GC column?

Pyrolysis is being used.

With respect to oxygen content @ altitude, the percentage of O2 remains the same i.e 20.9 %.

But the actual amount of O2 decreases BUT THE PERCENTAGE IS THE SAME !!

Calibrate O2 meter to 20.9% no matter where you are...

Lets try aromatic essential oil (tea tree) in MEK. We all that ketones are borderline w/ GC limit we have. 2 strong peaks w/ MEK. It is usable.

How to get clear of a strongly tailing peak?

1. Try essential oil
2. Determine CH ratio if possible
3. Letter out

We are now trying an essential oil for the first time in GC with the Sep D column. Rather exciting, I must say. It will be about an hour run w/ a ramp from 150 to 220°C. Good baseline @ elution time.

I have a beautifully clean additional peak w/ tea tree oil occurring @ ~10.5 min @ 150°C.

The detection of tea tree oil compounds is superb. Very good clean work with GC going on here, my first time w/ a solvent. The yeast starts to open up the GC work. Ideally molecular weight < 300 in the GC column.

There are three ingredients of tea tree oil

1,8 Cineole	$C_{10}H_{18}O$	(Eucalyptol)
γ-Terpinene	$C_{10}H_{16}$	
Terpinolene	$C_{10}H_{16}$	

We basically have a C_{10} Compound likely coming @ 10.5 min @ 150°C in the Sep D Column. There is great Next comes the broad tailing 2nd MEK peak, then one is not helpful to the cause.

It is extremely beneficial to have parallel comparison of Chromatograms for real time in Peak Simple. Any changes can be directly compared.

We believe $C_{10}H_{16}$ Compound has eluted since there is certainly baby plant. Very clean baseline.

I notice on GC substantiated chart from Vikki Dolan that polymer monomers are acceptable for in GC. Now it seems to me that when you are dissolving styrofoam in MEK that you have likely reduced the polymer to just that, monomers.

Let run on Column @ 220° to see if we can pull out the polar compounds w/ in the tree oil.

We now run @ 220°C
We have the air peak and the MEK 1st peak both within 2 minutes.

Something of interest here is that it looks like the trap of H_2O has collected material where is highly non polar. I would think that both the HC Tr HC seems to have eluted also w/ in 3 min.

We now get the 2nd Polar MEK peak coming out in about 5 min (tracing). Short delivery and jammed w/ in 1 min.

There are 3 things you can do w/ a TCD GC to increase the sensitivity, if required.

1. Decrease the temperature
2. Decrease the flow rate
3. Increase the current to the TCD.

We have a small peak @ 29 min w/ the Tea Tree - Very small.

The trap does not contain an insoluble; it was only a paper label.

You did not extract any second component from tea tree oil @ 220°C for 40 min. Either there is no other component (ie, primarily or only Eucalyptus oil, in it will not elute from the Column properly.

The question I have now is does MEK reduce styrofoam to a monomer? It would appear it does the same but can it harm the column?

With the styrofoam in MEK experiment it looks like I have a degrade peak addition, similar to what happened with tea tree oil.

Tea Tree Oil Compound is $C_{10}H_{16}$
Styrofoam is $(C_8H_8)_n$ 10.62 min

So they are indeed reasonably similar. I need to repeat tea tree experience I cannot find the file. My notes say the tea tree also had a new peak near 10.5 min.

MEK + styrofoam produces a peak also near 10.5 min?

A mystery has occurred that will take some time to settle.

1. MEF Control looks valid & repeatable w/ 2 significant peaks + the air peak.

2. BHT Tea tree oil AND Styrofoam are producing an identical peak @ 10.62 min. How can this possibly be?

Two completely different compounds producing an identical peak?
Each run take 1 hr to complete.
But we can drop it to 35 min @ 150°C.

Styrofoam has a secondary peak @ 13.90 min that tea may not?

Styrofoam is now in process again.

I just ran Styrofoam has a small peak @ 2.87 min. That has been confirmed twice.

The file name does not change as I am saving it. There is oddly consistent. Styrofoam is running on Chemstation.

Then tried the styrofoam peak is much weaker. As apparently I did not add as much

Let's run these @ 220°C. It may perform differently and will take less time.

This is perplexing. What are the chances on any given run of Sunday identical peaks for different substrates?

The styrofoam peak on this run is much weaker than the last, which makes sense.

220° is much more straightforward than Cast to work with. Peaks are easily separated.

MEK w/ styrofoam: Our new peak is @ 2.92

MEK w/ Tea Tree Oil: Our new peak is @ 2.89

This is remarkable to me. It demonstrates that peaks can not @ all be assumed & be unique where in the books all the time. A classy example.

I do think that this is bazaar. What are the chances of this?

There are some differences in the way the 2nd MEK peak is trailing.

The file names in Peak Simple are not updated when the file is saved. Only when they are retrieved and opened.

OOPS. We have a little problem here. MEK, by itself in products, the peak @ 2.06 @ 220°C. I have no idea why we did not see it originally.

Then under everything and to say that WE WERE NOT successful in extracting a peak from either the essential oil or the shyfoam. That is not a good result and it is highly disappointing. That means that items are still locked up in the column and that Co 9 : Co 10 were indeed too difficult to get out of the column. That is an important lesson.

Also we see that MEK is much too busy in the background to use as a solvent. OK, we do have problems. The MEK is actually composed of 3 different parts, that is noted on label from store.

MEK is hardly pure, therefore. Too bad.

Well, this was certainly a big lesson on an introduction to solvents.

1. Pure, suitable solvents are going to be hard to come by.

2. C_6, C_{10} compounds are not going to elute from the column, even under your higher temperatures.

3. You have to learn about derivatization to proceed w/ the furaken.

4. Gases still remain your best option w/ GC, including pyrolysis. Pyrolysis might be unique but it is most identifying. It does, however, offer the potential of a signature for solids.

5. Your next best bet, besides progress with

   1. Gas analysis
   2. pyrolysis analysis
   3. Is Elemental Analysis - can you get the CH ratio?

   4. Conceivably, you might be able to do trace solvent analysis.
   5. You must study derivatization to go further.

We know the SEP D can go to 290°. Therefore:
Oven? SRI says 300°C Filament 255°
TCD? SRI says 275°C Oven 250°

I have increased an oven and TCD configuration now to 250°C.

I have set oven max @ 250°C
I have set TDC @ 255°C
(there is the weakness to always be @ the max temp).
The column says that it is rated to 290°C.
You could adjust TCD between 220-250 depending upon plans.

You should do a study on essential oils for example. You are after low molecular weight components these just the name of the same.

It appears easy to detect solvents of solvent mixture but what I want are trace materials WITHIN the solvents. The does not appear so accessible.

Guess what: The MW of tea tree oil is 716

No wonder it is not passing through.

Zinc
$C_{28}H_{60}O_4P_2S_4Zn$

BP is ~165°C

Back to combustion analysis – Univ of Idaho
Start again w/ a simple hydrocarbon

$C_xH_y + n_0(O_2 + 3.76N_2) \rightarrow n_1(CO_2) + n_2(H_2O) + n_4(N_2)$

In and out.

We should be able to determine %O_2, %N_2, & CO_2

C: $x = n_1$

H: $y = 2n_2$

O: $2n_0 = 2n_1 + n_2$ (with no sulfur produced)

N: $2n_0(3.76) = 2n_4$ or $n_0 = n_4/3.76$

Assume we measure O_2 Input air = measure Output N_2 = measure = .47

O_2 output air = measure. ~~To determine the~~

measure CO_2 = .08

$C_xH_y + .125(O_2 + 3.76N_2) = .08(CO_2) + n_2(H_2O) + .47(N_2)$

Those coefficients do not add up to 1.00

This leads to $n_2 = 0.09$ See if this all balances

$2n_0 = 2n_1 + n_2$

$n_2 = 2n_0 - 2n_1 = .25 - .16 = .09$

~~$+ .125(O_2 + 3.76N_2)$~~

$C_{.08}H_{.18} \rightarrow 0.08(CO_2) + 0.09(H_2O) + 0.47(N_2)$

Notice $n_1 + n_2 + n_4$ do not need to add to 1.0

Jul 18 2017

Let's regroup on projects:

[redacted]

2. Combustion reaction & elemental analysis are absolutely fundamental skills to develop. With O2 metrics we should be in a better position now.

3. Fundamental skill development w/ GC is an equally valuable skill set
   1. HC's are in place
   2. low molecular wt limitation accepted but not appealing
   3. Pyrolysis work - unique signature? Gas comparison
   4. Headspace method tried
   5. Elemental analysis
      Stoichometric w/ GC based (this did not work out yet)

4. DNA increased production is on tap

5. Citizen sample evaluated

6. What are the specific chemical, biochemical & laboratory needs right now? Are there any of already importance beyond DNA?

7. Gamess analysis - this doe show some serious simulations, it seems

8. ICML release
9. Davis course (x)
10. Proline - Glutamic acid - Tryptophan simulation
11. Microwave digestor study
12. Brain wave study
13. Electrochemistry (exp. in lab)
14. Candle production of MO Cu?
Calibrate the Hayd dep 0 C 150°C.
15. Fish oil in GC in alcohol or acetone? (Triglycerides)
16. We have ethyl acetate!

I think that we should burn sugar to see what we can learn via GC.

heat-rise

Temp on edge of pan: 290°C → 300°C → 303°C $\bar{X} = 300°C$.
O_2 reading: 20.8 → 18.9% → 19.4% $\bar{X} = 19.15$
Trap at 40 torr on TCD.

We notice we are getting numerous peaks w/ the pyrolysis / combustion of sugar. Somewhat unexpected how active this is.

Our focus will be on N_2, O_2 & CO_2 free at low.
We will eventually desire all peaks.
We have 13 peaks w/ sugar alone w/in < 20 min.

You will eventually compare mixture to charcoal powder & determine relative ratios or contributions of components. Abel peaks will need to be identified.

It would certainly be nice if one of these peaks were water.

There is back pressure in the column. This indicates that it may be too high.

We have 6 peaks above w/ held human breath. This is amazing from a clean balloon. Mass Spectrometer is required.

Now we try a water vapor test.

Water vapor is indeed showing up w/ an unusual peak shape.
The balloon tube, however, is contaminated.

2nd run we seem to have a clean H_2O peak. This is invaluable. In the process you have discovered a way to clean the combustion tube w/ water bombs in tube.

You would have had lots of contaminent in on your previous run that fouled due to the results.

This is marvelous. The Hayes Sep O is going to give you a water peak, albeit somewhat trailing. This is going to be invaluable.

We should therefore be able to get a CO_2 peak & a H_2O peak which actually should be all that is required to obtain a CH ratio. Oxygen input & output would only help matters.

With water in the test tube and heated we measure .. to

81.5% $N_2 + O_2$	Ø.61
21% O_2 input & output approximate	~~Ø.92~~
Ø.78% CO_2	Ø.92
15.0% H_2O	1.57 L43 Trailing

This is very cool. We are definitely getting a straight readout w/ decent peaks. by N_2+O_2 - CO_2 & H_2O. We should be able to investigate the CH ratio.

For sugar, we have:

$N_2+O_2 = 64.7\%$

$CO_2 = 29.7\%$

H_2O 3.06%

Now let's set up the Combustion reaction.

Let's leave Oxygen out for now. (?)

$C_xH_yO_z + n_0(O_2 + 3.76N_2) \rightarrow n_1(CO_2) + n_2(H_2O) + n_4(O_2) + n_5(N_2)$

$X = n_1 = .297$

$y = 2n_2$ or $y = 2(.0301) = .0612$

This means

$C_{.297}H_{.0612}$ HC Ratio = ~~.206~~ .206

NO there is a problem

MW Sucrose is $C_{12}H_{22}O_{11}$ HC Ratio = 1.83

This is ~~not bad~~ already!

What about response factors

Clearly we have already identified an ~ 2 to 1 CH ratio almost immediately

That's fantastic

		Response Factor	Wt. Factor
MW CO_2 =	44	48	44/48 = .917
MW H_2O =	18	21	18/21 = .857
MW O_2 =	32	40	32/40 = .800

~~Therefore O_2~~ O_2 .1239 (.800) = .991

CO_2 : .297 (.917) = .272

H_2O : .0301 (.857) = .026

~~1.298~~ Σ = 1.289

CO_2 $\frac{.272}{1.289} = 21.1\%$ by weight? .272 / 1.289 = 21.1% ... 5.76% is Carbon

H_2O $\frac{.026}{.298} = 8.7\%$ by weight ... 8.97% is H

O_2 $\frac{.991}{1.289} = 76.9\%$ by weight ... 100% is O

So clearly you have a problem. You missed a decimal point.

The older approach:

C: ~~29.9 gms~~

No, No, No, you cannot do what you have done.

~~CO_2 % = 29.68~~

~~29.68 +~~

What you actually have is

O_2: (.1915) 64.71% = 12.39% O_2 100% is O

% O_2

CO_2: 29.68% 27.3% is C, 72.7% is O

H_2O 3.06% 11.2% is H, 88.8% is O

Now:

$X = n_1 = .297$

$Y = 2n_2 = 2(.0306) = .0612$

$Z = 2n_1 + n_2 + 2n_4$ and $n_4 = .124$

So $Z = 2(.297) + .0306 + 2(.124) = .873$

leads to

$C_{1.297} H_{.0612} O_{.873}$ but it should be $C_{12} H_{22} O_{11}$

Mass % is C: 43.5%

H: 3.35%

O: 53.1%

$\Sigma = 99.9\%$

Well, we clearly have some problems.

The combustion gas may not be all compose with that produced by the combustion chamber.

Let's try that gas.
In our second run, w/ the copper combustion chamber, we have quite a different situation.
We have

% Area:		Area	MW	Response	Wt
	$O_2 + N_2$	103.28			
1.63%	CO_2	0.41	44	46	.917
12.20%	H_2O	3.07	18	21	.857
86.17%	$O_2 = 21\%$	$= \frac{21.69}{25.17}$	32	40	.800
	$N_2 = 79\%$	= 81.59	28	42	0.67
		Total 106.76			

$1.63(.917) = 1.49 \quad = 12.5\%$ but $27\frac{1}{3}$ is C $\neq$ 3.41% C

$12.20(.857) = 10.46 \quad = 87.5\%$ 11.2 is H $\neq$ 9.80 is H

$\Sigma = 11.95$

Ratio is 2.88 to 1
Should be 1.86 to 1

This is supposed to be ratios of weights

C: $3.41(.2729)/12.011 = .077$ Ratio = 14.5 to 1

H: $9.80(.1149)/1.0079 = 1.09$

Ratio should be 1.86 to 1.

The says too much water, not enough carbon.

What about your simple combustion tube?

There are definitely problems w/ GC methods combined w/ combustion analyses.

I have tried 3 different methods and the results vary accordingly. There seems to be no systematic w/ the errors.

Case 1: Balloon method. Good looking peaks. High CO_2, low water outputs

Case 2: Copper combustion tube. High water, modest CO_2

Case 3: Simple combustion tube. Very high water, low CO_2

What should the results be?
Moles of H to moles of C should be in ratio of 1.86. The same as in our original copper combustion chamber work.

The problem w/ GC & combustion analyses appears to be that combustion is not a snapshot process, it would seem to be an integral process with changes (esp H_2O content) occurring continuously & what matters is the total accumulation of combustion products throughout the reaction. Thus the clamor for CO_2 & H_2O "traps" - you need it all over time.

Assuming CO_2 area & H_2O area comprise the only peaks of interest (ie Σ area = 100%).
Then:

$$\frac{A_{CO_2} \cdot Wf_{CO_2}}{A_{CO_2} \cdot Wf_{CO_2} + Wf_{H_2O} \cdot A_{H_2O}}$$

(this is our 1.2729)

$\cdot$ 27.3% (portion that is C) = Carbon Area

Relative

(this is our 1.119)

and similarly for H_2O $\cdot$ 11.2% (amt that is H) = 1.86 $\cdot$ Carbon Area

Relative

If your work is in grams, you need to divide by grams/mole to get the result in moles.

Review Case 1 : Balloon Combustion Tube
Clean Peak Formation

Page 190

		MW	Response wt	
CO_2 Area	29.68	44	48	.917
H_2O Area	3.06	18	21	.851
	Σ = 32.74			

On water peak should be 4 times larger.

		Weight %	Assume 100 gms
CO_2 :	29.68 (.917) = 27.22	91.2	91.2 gms
H_2O :	3.06 (.851) = 2.62	8.8%	8.8 gms
	Σ = 29.84		

Now gms of C = 91.2 gms (.273) = 24.90 gms C
gms of H = 8.8 gms (.112) = 0.986 gms H

So no of mole C = 24.90/12 = 2.075
no of mole H = .986/1 = .986

and the ratio of H to C is .475 but it should be 1.86 to 1

This is the heart of our problem. The H_2O peak should be about 4 time larger.

What is the sucrose combustion reaction?

$C_{12}H_{22}O_{11} + 12O_2 \rightarrow 12\,CO_2 + 11\,H_2O$

Take a look closer @ this, this is in range

CO_2 91.2 gms/44 = 2.07
H_2O 8.8 gms/18 = 0.49

~~This is not possible~~. (yes it is)

Ratio should be $\frac{12}{11} = 1.09$

This is actually within range

We have $\frac{2.07}{.49} = 4.22$

off by a factor of 4.

The integral vs snapshot assessment appears to be a critical issue and getting repeatable & accurate results of CH_4 ratio appears to be difficult, if not impossible, via GC.

You may be restricted to the stoichometry approach here. The problem could be affected by oxygen results as well; it (the combustion) is probably always changing.

You have also learned the problem of contamination & crossover bleed in the combustion tube when changing from one sample to another. Spare tubes would be helpful to allow cleaning and drying of tube while switching to an alternate tube.

Notice the high water content of the short & simple combustion tube as opposed to the low level of the elevated balloon combustion tube. So how would you know which is correct? You don't. You must collect on over all vapors over time & through the CO_2 & H_2O traps.

It was, however, a very good try.

Acetone makes a good clean solvent peak on Hayes Sep D @ 150°C. Possibilities here.

No "anaerobic" a "reduced O_2" culture!

Page 1/92

I think that you have put forth a good effort w/ GC. & just do not think that it is going to work w/o the ability of the GC to accumulate & capture that over time.

The O_2 meter is still a very helpful addition. You can use that in many ways. For instance, what is the O_2 of the balloon culture? How valid is your assumption of a "restricted O_2" culture?

Let's see.

Guess what? There was no reduction whatsoever in the O_2 content w/in the balloons of the capped cultures. The idea of an "anaerobic culture" or a "reduced oxygen culture" is * A MYTH. It is not the case and all use of those terms must be stricken completely from the record. *

You must only refer to this culture vibration as a "trapped" culture (ie, balloon) subject to extended incubation. We have learned that both hydrocarbons & protein are produced in the process, both of these being of monumental importance in terms of the prospect and evidence for genetic engineering.

The O_2 meter has already proven its value therefore, both in N_2-O_2 separation & w/ the culture asmt.

Jul 19 2017

Is there any chance that there was a bleedover Contamination issue w/ the HC determination on the COB "hap" culture?

Lets try to repeat. It is questionable whether the culture are sufficiently developed or not. Test w/ mixed min Controls w/ propane & butane

Lets start calibrating the Hayes skpD @ 150°C
We have

	N_2-O_2	0.63	MW Middle for CN & MW
	CO_2	0.91	are logarithmic
	H_2O	1.52	
3	Propane	~ 2.17 2.4	44
4	Butane	5.36	58
2	Ethane	1.51	30
4	Butene	6.16	56
3	Propene	2.65	42

But we know that many hydrocarbons ARE being produced because of the pyrolysis of the charred proteins. We have made it at least to pentane w/ everything along the way.

Actually we have a mix of hexane and signs of higher activity as well, beyond the column capability.

$CN \cong 1.40 \ln(t_{150}) + 1.6$ $r^2 = 0.97$

$t_{150} \cong 2.28 \cdot CN - 3.4$ ~~$t_{150} \cong 2.28 CN - 3.4$~~ $t_{150} \cong 2.20 CN - 3.4$ OK

$MW \cong 18.95 \ln(t_{150}) + 24.2$ $r^2 = 0.95$

$t_{150} \cong 0.17 MW - 4.0$

Not bad for first models.

CN		for CN
5	Predict Pentane: MW = 72 $t_{150} \cong 8.2$ min	8.0
6	Hexane: MW = 86 $t_{150} \cong 10.6$ min	10.3
7	Heptane M = 100	

OK, you already have estimates for t_{150} for C5 & C6

C5: $t \cong 8.0$ min good

C6: $t \cong 10.7$ min

C7 $t_{150} = 12.5$, $t_{150} = 13.0$ $t = 12.8$ min

What happens with a protein like milk? Wool? Blood? yeast?

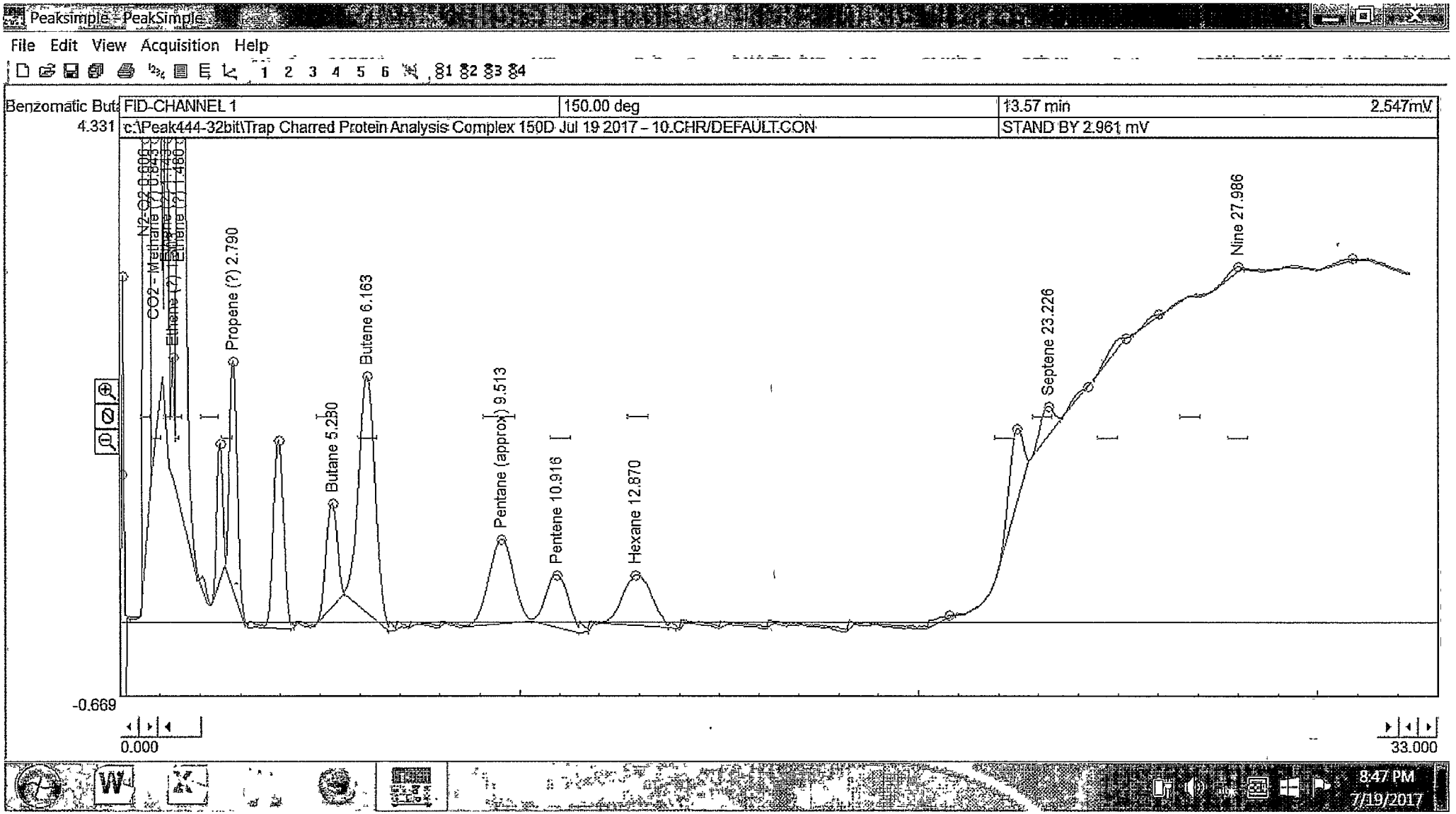
C2	= 2			
C3 (2)	6			
C4 (2)	8			
C5 (2)	10			
C6	6 H's	⇒ C_{42} min	if saturated $C_{42}H_{86}$	MW = 590
C7 (2)	14	= C_{49} min	$C_{49}H_{99}$	MW = 690
C8 (2)	16 O	C_{57} min	$C_{57}H_{116}$	MW = 800
C9 (2)	18	$C_{80}H_{162}$		MW 1122

We have major hydrocarbon products confirmed from pyrolysis of the Charred pellets. We have estimate of C100+ now.

Our model can be improved significantly from this result.

This is the highest level of separation ever achieved this far. Excellent work here.

CDB Hydrocarbon Analysis Jul 19 2017



Jul 20 2017

Today, primary goals are the pyrolysis of powdered milk and of yeast.

I have started w/ milk. The results are again highly successful w/ clean results.

I am easily up to septane now and most likely even higher, as with occurred w/ CDB cleaved protein. Milk pyrolysis is very complex and thus is great as pyrolysis is obviously acting as a signature-spectral method as well here.

What is the approx molecular wt of powdered milk, eg Casein?

You have a strong peak even at t= 27 min @ ramped 220° segment.

Profile is 20 min @ 150°C
Ramp @ 30°C per min to 220.
Hold @ 220 for 20 min.

Repeatability of pyrolysis is going to be a very interesting topic.
We estimate that we are operating between 400-500° during the sampling process. (Balloon w/ syringe puncture on top of test tube to collect gas).

Page 198

We also wish to use our model to include some of the higher JHC's.

Our next step is to investigate the repeatability of the powdered milk pyrolysis. Technically a new sample should be used but I would like first to investigate the remaining sample material (partial combustion).

The location of peaks is the same but the magnitude is affected. It will be better for consistency to use a fresh sample on each occassion if at all possible.

It will be of interest to examine the case of it being known that one of the primary products from the pyrolysis of Glutamic acid (one of the amino acids identified as strong candidates) in COBS secreted protein analysis is Propene.

Proline produces pyrrole (what is this?)

Changing the column temperature does wreak havoc in the equilibration process. It would be best to hold mat work @ 150°C for about 30 minutes, and then ramp 220 times as a separate process if need require a bakeout at final run.

We can see that the (milk) pyrolysis is quite reproducible in general. It can be seen that a second sample reached a higher temperature

but that the structure of the peaks is highly coincident between the two runs. There is indeed a valuable spectral representation here that is of value in establishing a separation profile of pyrolyzed components.

Now lets go back to protein (Casein) for comparison to milk.

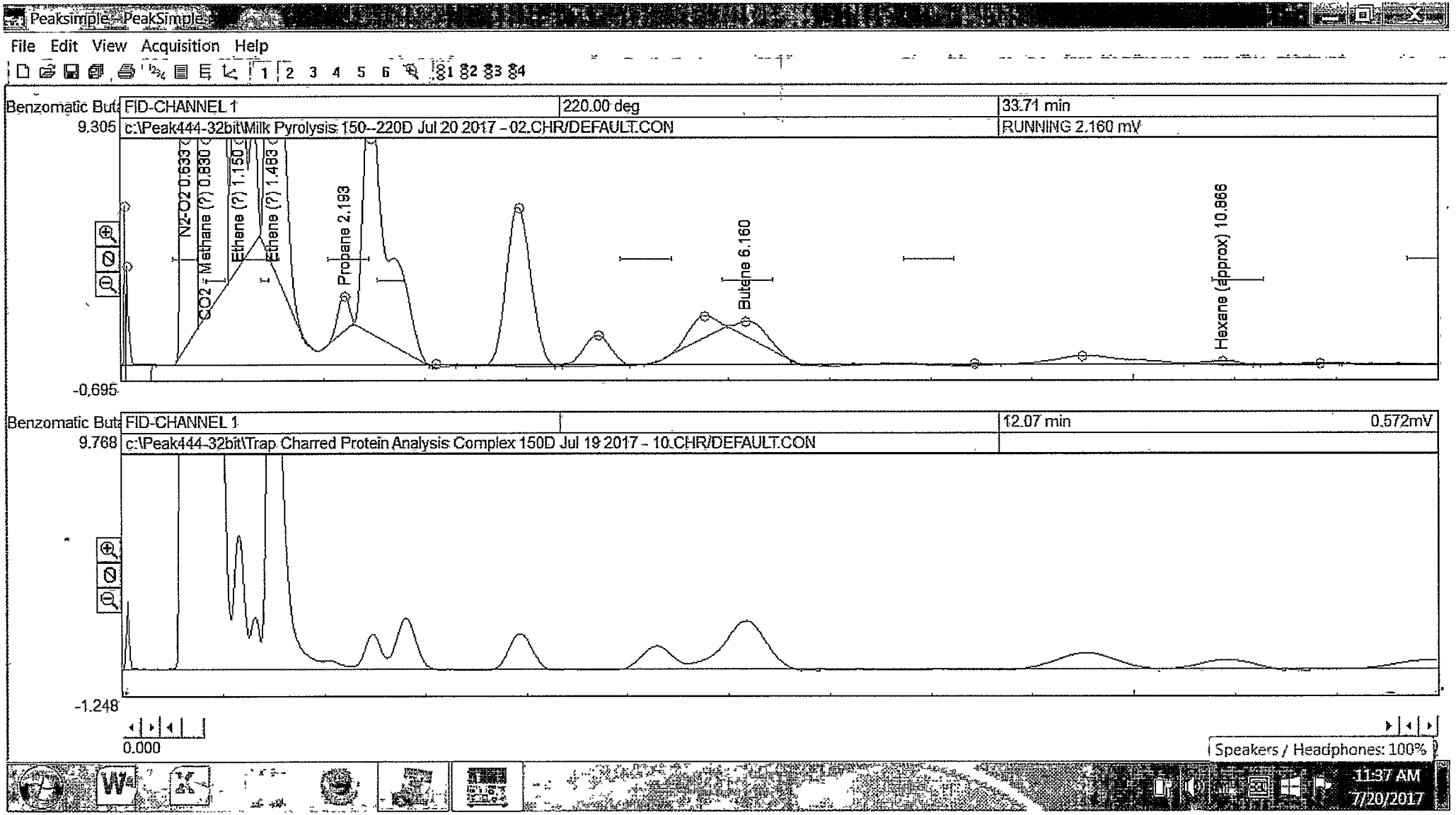
We see that, although there several important similarities (especially in the low end retention times) they also vary a fair amount between 2-20 min @ 150°C. They may contain to vary between 20-30 min. Let's test this.

Casein has a molecular weight of ~ 800 It is C36. There is right in range of what I would anticipate from the milk pyrolysis and in range of what we deduced from the accumulation of pyrolytic components also w/ the CDB protein.

Conclusion: Powdered milk pyrolysis result is quite distinct from that of CDB secreted Protein, like for yeast.

Page 198A

Powdered Milk Pyrolysis compared to CDB Hydrocarbon Analysis Jul 20 2017



Jul 21 2017

We have been able to measure the temperature of the pyrolyzer chamber. The maximum achieved was ~ 600°C.

Our estimate of reaching a temp range of 400-500°C on average was quite accurate.

I believe ~ 500°C is our central avg temp reached in our chamber. Lower heating will approach ~ 400 and max heating will approach 600°C. The is indeed right about where we should be.

The upper portion of the chamber cools off very quickly, it can easily be 100-200°C cooler than the sample.

The pyrolytic process is regarded generally as a suitable "fingerprint" method for establishing uniqueness of a sample.

Let's develop our model in the Hayes Lab D today.

I believe it is possible for me to tap output from the GC into a hydrogen and feed it into the IR gas sampler. This would be a huge benefit.

We will establish a control run w/ propane first to get the retention time window

We know it is about 1.0 min
Ok, easy to capture on the first run.
Let's see how we do.
I got it on the first pass.

This is fantastic. I also have a funky stray signal.

You notice that it disturbs the chromatogram because of the vacuum pressure (you are drawing in on the syringe @ the appropriate time). But so what it does not have any thing and eventually the GC signal was strong enough to override the effect.

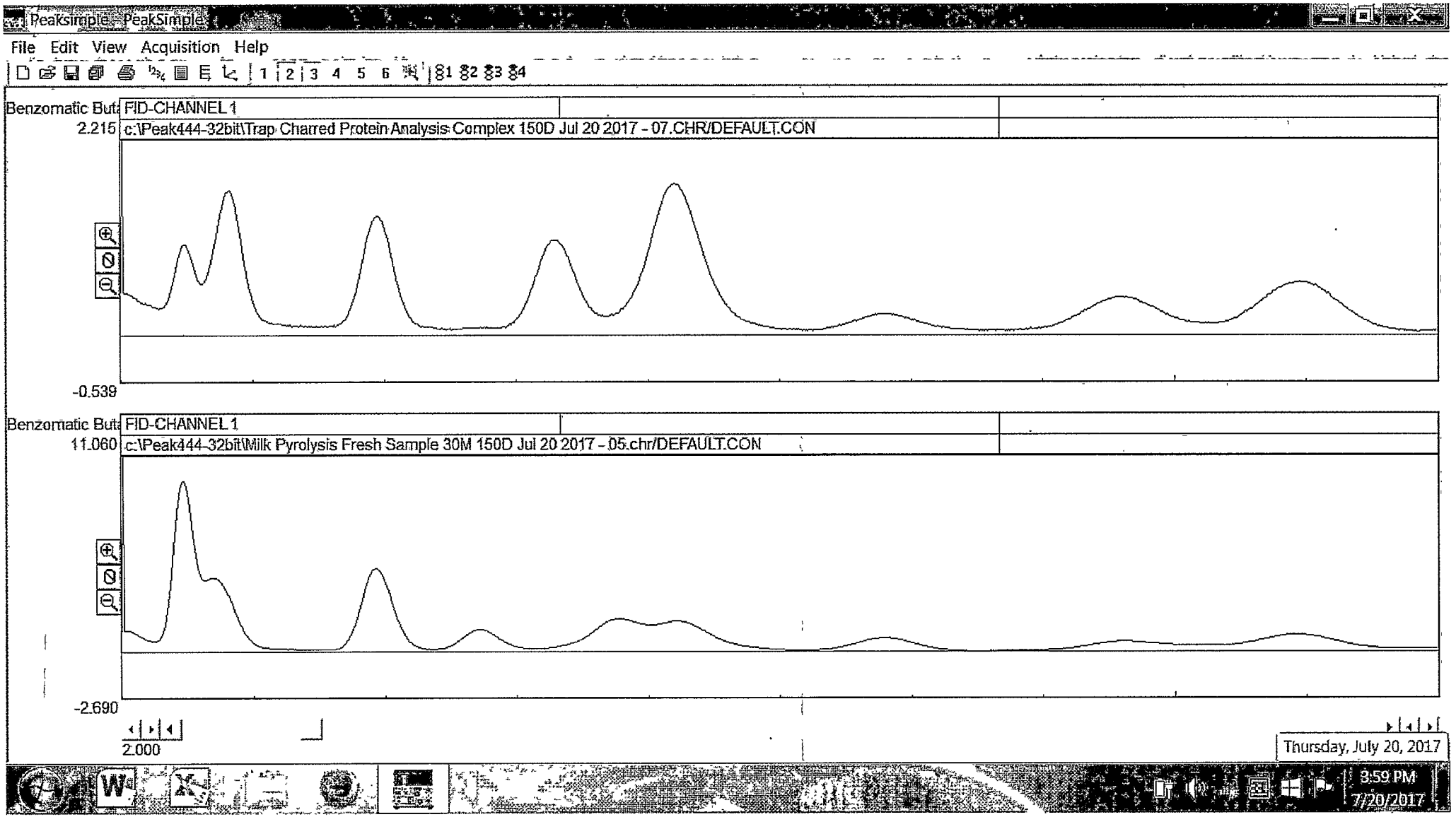
You got an absolutely pure alkane signal w/ propane first, this is the first time that you have seen this so purely. This is beautiful work that under the right circumstances, will both SEPARATE AND IDENTIFY (at least to functional groups).

We get peaks @ 2961
2899

We must also keep calibrating the GC work as it will help to identify unknowns and non HC's

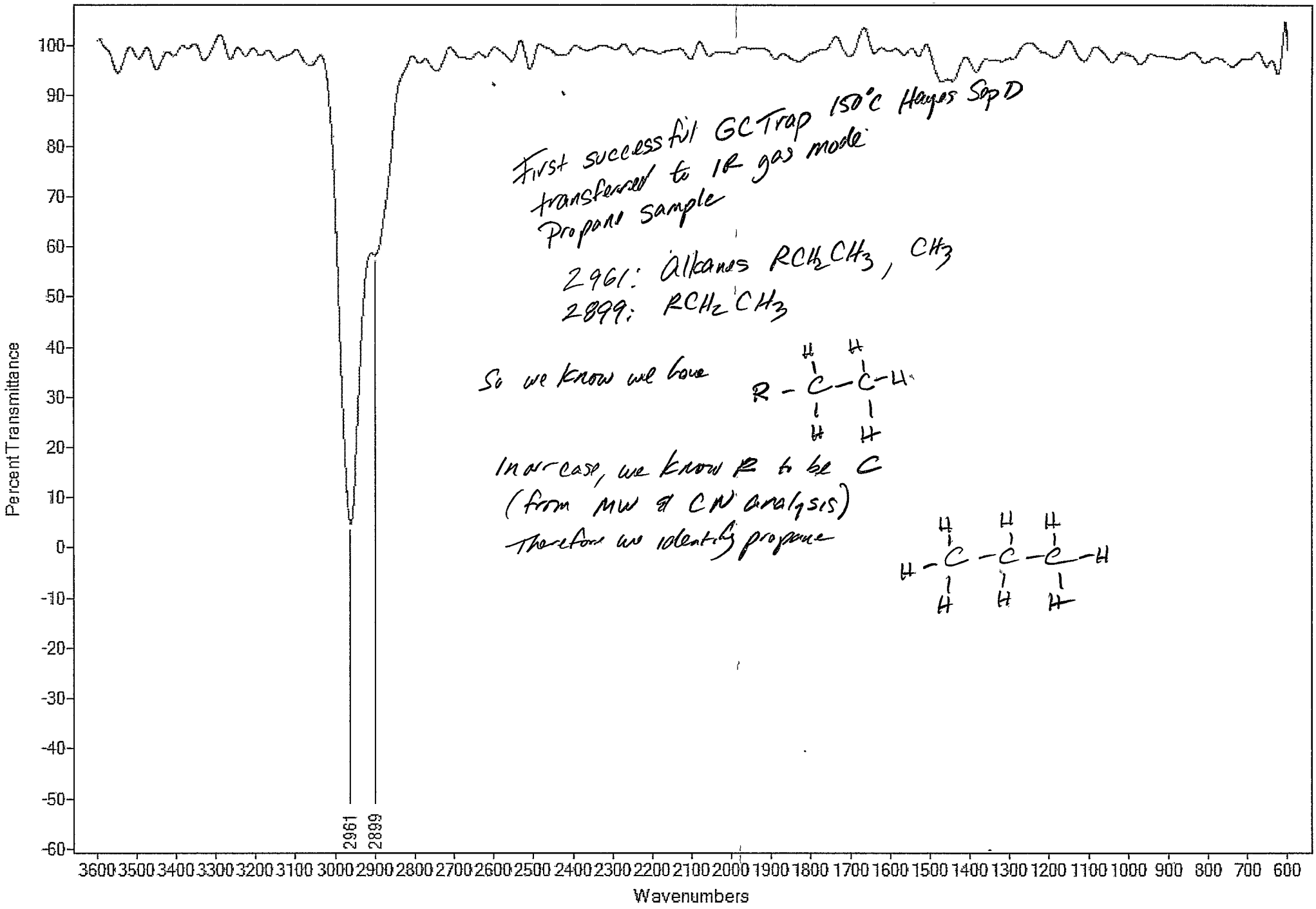
Pyrolysis Finger-print Signature of CDB
Secreted Protein & Comparism to Dried Milk
Uniqueness of Pyrolysis demonstrated

CDB Protein Pyrolysis compared to Dried Milk Pyrolysis Jun 20 2017



Successful GC Trap transferred to IR gas analysis:

Propane GC Trap Sample - Excellent Work Jul 21 2017 - 01.JPEG



First successful GC Trap 150°C Hayes Sep D transferred to IR gas mode
Propane sample

2961: alkanes RCH_2CH_3, CH_3
2899: RCH_2CH_3

So we know we have

In our case, we know R to be C
(from MW & CN analysis)
Therefore we identify propane

Next we need to look @ sensitivity of the method. The butane peak is fairly small, so it will make a good test.

We also see that the IR gas sampler is hardly airtight so we lose the signal within a few minutes. You must work quickly & efficiently w/ the process.

Lets try the butane-butene peaks.

These peaks occur from 4.5 min — 7 min
The good news is that by you have enough sample you can synchronize extraction-trapping from the GC.

A 2.5 min sampling interval is quite long. I suspect you can work w/ 30 sec very easily.

The signal is not detectable.

Propane had a GC peak ht of 260 mV
Butane has a peak height of 1.85 mV
Butene has a peak height of 0.9 mV

This tells us that our signal strength in GC has to be fairly strong to use this method, I will presume on the order of 20 mV or so.

So while it is not something that can be used for everything on the GC list it can be used for the stronger signals.

It is nevertheless extremely valuable and coupled with GC MW & CN estimates, can be a valuable assist in identification of compounds.

Next lets improve the Hayes Sep D 150°C model.

Page 207

CN		t	MW
	N_2–O_2	0.62	28–32
	CO_2	0.91	44
	H_2O	1.52	18
C2	Ethane	1.57	30
C3	Propane	~2.41 2.40	44
C3	Propene	2.65	42
C4	Butane	5.36	58
C4	Butene	6.16, 6.21	56
C5	Pentane	7.77 7.78 7.72	72
C6	Hexane	10.89 10.90 10.84	86
C7	Heptane	14.5	100
C8	Octane	16.5	114
C9	C_9H_{20}	18.7	128
C10	$C_{10}H_{22}$	21.8	142
C11	$C_{11}H_{24}$	25.1	156
C12	$C_{12}H_{26}$	28.1	170

Let's predict heptane:

$t_{150} \cong 2.46\,CN - 4.2$ $\quad r^2 = 0.97$

$CN \cong 0.39 \cdot t_{150} + 1.8$ $\quad r^2 = 0.97$

$t_{150} \cong 0.173\,MW - 4.4$ $\quad r^2 = 0.97$

$MW \cong 5.57\,t_{150} + 26.2$ $\quad r^2 = 0.97$

Now predict heptane: (t_{150})

$t_{150} \cong 12.90$ (MW prediction) $\quad \bar{t} = 11.8$

$t_{150} \cong 10.6$ (CN prediction)

Using Thought Butane only:

Try to Predict Pentane (t_{150})

$t_{150} \cong 1.92\,CN - 2.8$ $\quad r^2 = .90$

$CN \cong 1.54 \ln(t_{150}) + 1.48$ $\quad r^2 = 0.98$

$t_{150} \cong 0.138\,(MW) - 3.0$ $\quad r^2 = 0.91$

$MW \cong 21.62 \ln(t_{150}) + 22.2$ $\quad r^2 = 0.97$

$t_{150} = 6.8$ (CN) $\quad \bar{x} = 6.9$

$t_{150} = 6.94$ (MW)

Our measured value of 7.76 is valid therefore.

Now include pentane in to model.

$0.571 \cdot CN$

$t_{150} \cong 0.416\,e$ $\quad r^2 = 0.98$

$CN \cong 1.72 \ln(t_{150}) + 1.34$ $\quad r^2 = 0.98$

$0.040 \cdot MW$

$t_{150} \cong 0.459\,e$ $\quad r^2 = 0.98$

$MW \cong 24.34 \ln(t_{150}) + 20.0$ $\quad r^2 = 0.98$

Choose 10.9 min instead

Now to predict hexane:

$t_{150} \cong 14.64$ $\quad \bar{x} = 14.5$

$t_{150} \cong 14.31$

This is where our question is.

We do indeed have a small peak @ 14.59
We also have intermediate unknown peaks.

The large peaks @ 9.6 & 10.9
are these for the unknowns

also a small peak @ 12.1

These 3 peaks introduce uncertainty.

We have a peak 14.55
and the model predicts 14.5
Try to corroborate w/ other materials.
Lorby @ mall.

Yeast has a similar behavior.
We have a definite peak @ 14.55

But also @ 9.6, 10.9 & 12.1

So indeed if hexane is 14.55 what are
these intermediate peaks? I am not sure
that they are strong enough to pick up on IR.

Is it possible that 12 + 10.9 is hexane?
That is a large peak & is the most logical.

Page 210

I believe that it is logical that the large peak @ 10.9 is indeed hexane.
Let us act upon the assumption & separate the model.

$t_{1/50} \cong .512 \cdot CN - 0.568$ $r^2 = 0.98$
$CN \cong 1.91 \ln(t_{1/50}) + 1.2$ $r^2 = 0.98$

$t_{1/50} \cong .036 \cdot MW - 0.548$ $r^2 = 0.98$
$MW \cong 27.01 \ln(t_{1/50}) + 17.6$ $r^2 = 0.98$

Now predict heptane

$t_{1/50} \cong 20.4$ (CN)
$t_{1/50} \cong 20.0$ (MW)

$\bar{X} = 20.2$ min

predicted for heptane

There is a definite peak @ 21.6 min.
It is also well formed and of modest decreasing magnitude. It is reasonable.

But we also have a peak @ 14.5
and there is also reasonable for a more sensible progression. Let us assume heptane is the 14.5
An exponential function may not be the best here.
Linear appear better.

$t_{1/50} \cong 2.68 \cdot CN - 5.0$ $r^2 = 0.98$
$CN \cong 0.365 \cdot t_{1/50} + 1.9$ $r^2 = 0.98$

$t_{1/50} \cong 0.19 \cdot MW - 5.3$ $r^2 = 0.98$
$MW \cong 5.16\, t_{1/50} + 28.4$ $r^2 = 0.98$

Ok, these are now our last intermediate model.

The linear model explains how we are picking up so many compounds now w/ the Haya step D vs the silica gel column. Haya step D appears to be far superior for separation of HCs.

Let's predict octane now: C_8H_{18}

$C = 8$

$MW = 114$

$t_{150} = 16.4$ (CN based)

$t_{150} \doteq 16.4$ (MW based)

We have an extended peak that does come out @ 16.5

but the next clear peak is @ 18.7 min

how does this correlate w/ the model

$CN \doteq 8.7 \doteq 9 = C_9$ OK

$MW \doteq \doteq 125$ $C_9H_{20} = 128$

This looks to be superb.

Try C_{10}. We have a great, well formed @ 21.8

$CN \doteq 9.9 \doteq 10$ excellent

$MW \doteq 140$ $C_{10}H_{22} = 142$

Amazing but we seem to be up to C_{10} quite smoothly.

Hayes Sep D Saturated Hydrocarbons Model

We will now refine the model again.

$t_{150} \cong 2.74 \cdot CN - 5.3$ — This is based upon actual measurements for Cn to C12 — $r^2 = 0.996$

$CN \cong (0.36)(t_{150}) + 2.0$ — $r^2 = 0.996$

$t_{150} \cong 0.19 \cdot MW - 5.6$ — A super model for saturated hydrocarbons — $r^2 = 0.996$

$MW \cong 5.11\ (t_{150}) + 29.1$ — $r^2 = 0.996$

What we see here is that the Hayes Sep D column is producing a perfect linear response w/ the saturated hydrocarbons. This is marvelous and far superior to the silica gel column.

We have another peak @ 25.1, let's try it. This is all still @ 150°C!

$CN \cong 11.2 \Rightarrow 11$ also excellent

$MW \cong 160$ Actual = 156

We therefore have C_{11} now.

We have 1 more peak @ 28.1

$CN \cong 12.3 \rightarrow 12$

$MW \cong 175$ Actual is 170

We have C_{12} w/ 150° w/in 30 min. Amazing.

Refine the model again.

I never thought this GC was capable of producing C_{12} output but we have it.

Page 213

We could now calculate a few peaks to 220°C and we may be able to extend the HC's even higher.

Continued from Jul 22 2017:
Like ref. to meter some peaks (yeast)

	C	150°C	220°C	
N_2-O_2		Ø.61	.65	OK, next Heatings
CO_2		.84	.72	
Ethane		1.48	1.04	
Unknown		1.14		
Unknown		1.30		
Propane		2.42	2.04	
Butane		5.56	2.99	

$t_{150} \cong 2.08\, t_{220} - 1.Ø$ $\quad r^2 = .90$

$t_{220} = Ø.43\, t_{150} + Ø.7$ $\quad r^2 = .90$

We now begin predicting.

$CN \cong Ø.36(2.08\, t_{220} - 1.0) + 2.Ø$

$MW \cong 5.11(2.08\, t_{220} - 1.0) + 29.1$

	^	^	Best Match		Compound
t	CN	MW	CN	MW	
1.04	2.4	35	2	30	Ethane
2.04	3.2	46	3	44	Propane
3.00	3.9	56	4	58	Butane
5.16	5.5	79	4-5	72	Pentane

We should be OK to here now.

Jul 22 2017

Today we will put the column further @ 220°C
First step is to calibrate propane & butane
@ 220°C

	220°	vs	150°C
O_2 Ethane	0.68		.62
Propane	1.42, 1.32	$\bar{X}$ 1.37	2.40
Butane	2.15, 1.85	$\bar{X}$ = 2.00	5.36

First test, note of 220–150 relation using propane & butane

$t_{150} = 4.70\, t_{220} - 4.0$ $\quad r^2 = 1.00$
$t_{220} = 0.21\, t_{150} + 0.9$ $\quad r^2 = 1.00$

Then a few extra only.

C	$\frac{1}{(C.N.)}$ t_{150}	MW	$\frac{1}{(MW)}$ t_{150}	λ t_{150}	t_{220}	
2	0.18	30	.10	0.14	.92	Ok, the same
3	2.92	44	2.76	2.84	1.5	first guide.
4	5.66	58	5.42	5.54	2.1	Now run complex
5	8.4	72	8.1	8.2	2.6	sample activity
6	11.1	86	10.7	10.9	3.2	to make these
7	13.9	100	13.4	13.6	3.8	primary peaks.

Page 215

Continuing before the 220°C Haya model:
We now look closer @ peaks coming out @ 220°C
We have:

It appear that 150° will give a little separation ultimately than 220°C. 220°C close not look especially beneficial @ this time.

	Peak @ t	CN	MW	Best Match CN	MW	Candidate
	0.67					N_2O_2
	0.78	2.2	32	2	44	CO_2
*	1.00	2.4	35	2	30	Ethane
	1.37	2.7	39	2	28	Ethene?
*	2.09	3.2	46	3	44	Propane
	2.56	3.6	51	3	42	Propene?
*	3.00	3.9	56	4	58	Butane
	3.59	4.3	62	4	56	Butene
*	4.23	4.8	69	5	72	Pentane
	5.16	5.5	79	5	70	Pentene?
*	6.29	6.3	91	6	86	Hexane
*	7.50	7.2	104	7	100	Heptane
*	8.92	8.3	119	8	114	Octane

Hayes Sep D 220°C Saturate Model

You have good general agreement here w/ unknowns @ $t_r = 1.37$ & $t_r = 2.5$ b
Ok, I believe these are resolved.

Ok, now we form new models for CN & MW based upon saturates from C2–C8.

220° model

$CN \cong 0.73\, t_{220} + 1.5$
$t_{220} \cong 1.35\, CN^{1.5} - 2.0$

$MW \cong 10.24\, t_{220} + 23.7$
$t_{220} \cong 0.10\, MW - 2.2$

220°C Sep Hayes model $r^2 = 0.99$
for saturates $r^2 = 0.99$
$r^2 = 0.99$
$r^2 = 0.99$

We should now have a reasonable 220°C Hayes Sep D saturate model.
Now look for the relationship between t_{150} & t_{220}.

C	t_{CN}	t_{MW}	t_{150}	t_{CN}	t_{MW}	t_{220}	
2	0.18	0.10	0.14	.70	0.80	0.75	Do not use this entry
3	2.92	2.76	2.84	2.05	2.20	2.12	
4	5.66	5.42	5.54	3.40	3.60	3.50	
5	8.40	8.08	8.24	4.75	5.00	4.87	
6	11.14	10.74	10.94	6.10	6.40	6.25	
7	13.9	13.4	13.6	7.45	7.00	7.62	
8	16.6	16.06	16.33	8.80	9.20	9.00	

$t_{220} \cong 0.50\, t_{150} + 0.65$ $r^2 = 1.000$

$t_{150} \cong 1.99\, t_{220} - 1.30$ $r^2 = 1.000$

$t_{150} - t_{220}$ Relationship

We now have enough information that we can reasonably equate for saturates:

1. Equate $t_{150} \leftrightarrow t_{220}$ on Hayes Sep Data

2. Predict or estimate a saturate for a given t on 150°C column

3. Predict or estimate a saturate for a given t on 220°C column

---

I wonder if there is any way that the $t_{150} \leftrightarrow t_{220}$ relationship can be predicted. I do not see it now.

Notice the slopes are roughly 4 to 1.

and though $\frac{220}{150} = 1.47$

Notice $e^{1.47} = 4.35$

There is an exponential relationship here of close or anything.

$$f\left(\frac{150}{220}\right) \stackrel{?}{=} \frac{1.99\, t_{220} - 1.30}{0.50\, t_{150} + 1.65}$$

$$Ae^{\frac{220}{150}} \approx \frac{d(f_{220})/dt}{d(f_{150})/dt} \quad \Rightarrow Ae^{1.47} = 3.98$$

$A \approx 0.91$

Would be of interest to test the rule $C^{\frac{150}{60}} \stackrel{?}{=} 6.5$?

What we see is that the best results are still achieved @ 150°C.

$t_{150} \cong 2.74\,CN - 5.3$ $\quad t_{150} \cong 0.19\,MW - 5.6$

$CN \cong 0.36\,t_{150} + 2.0$ $\quad MW \cong 5.11\,t_{150} + 29.1$

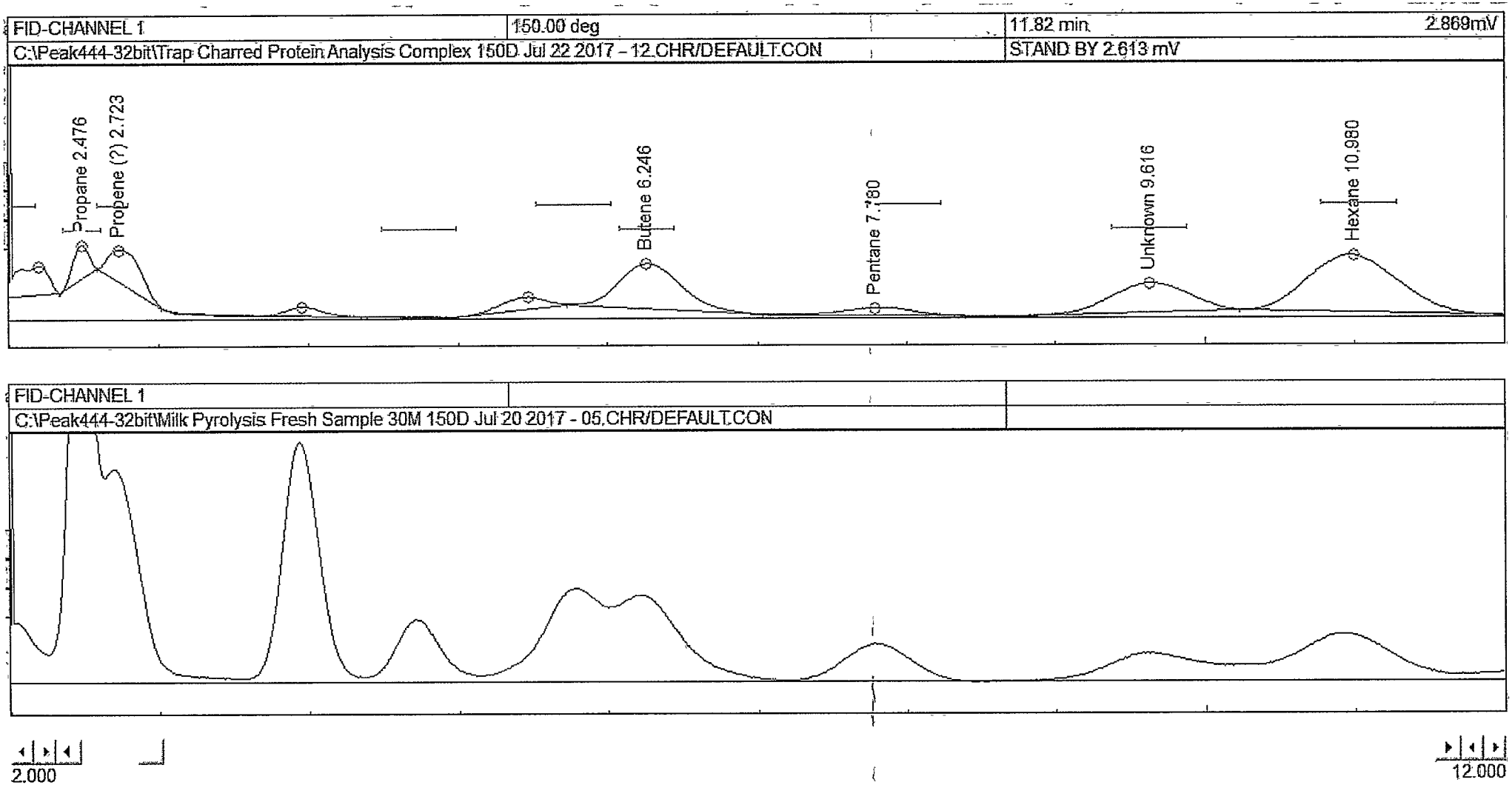
Peaks (t)	CN		MW		
					Yeast Analysis:
0.61					
Intermediate					
					Candidate
0.86	2.3		33	30	"" "CO2"?
1.16	2.4		35		Ethane
1.30	2.5		36		Ethene?
1.49	2.5		37		
2.06	2.7		40		
2.41	2.9	3	42	44	Propane
2.68	3.0	3	43	44	Propane, Propene
4.72	3.7		53		
5.80	4.1	4	59	58	Butane
6.29	4.3		61		Butene?
7.82	4.8	5	69	72	Pentane
9.65	5.5		78		Pentene?
10.97	5.9	6	85	86	Hexane
12.27	6.4		92		Hexene?
14.64	7.3	7	104	100	Heptane
15.63	7.6		109		Heptene?
17.97	8.5		121	114	} Octane?
19.52	9.0	9	129	114	}
GOB Chard:					
24.82	10.9	11	156	156	C11
28.8	12.4	12	176	170	C12

Page 219

Comparison w/ Dried Milk. – "Fingerprint" Acquired

CDB Secreted Protein - Pyrolysis Analysis

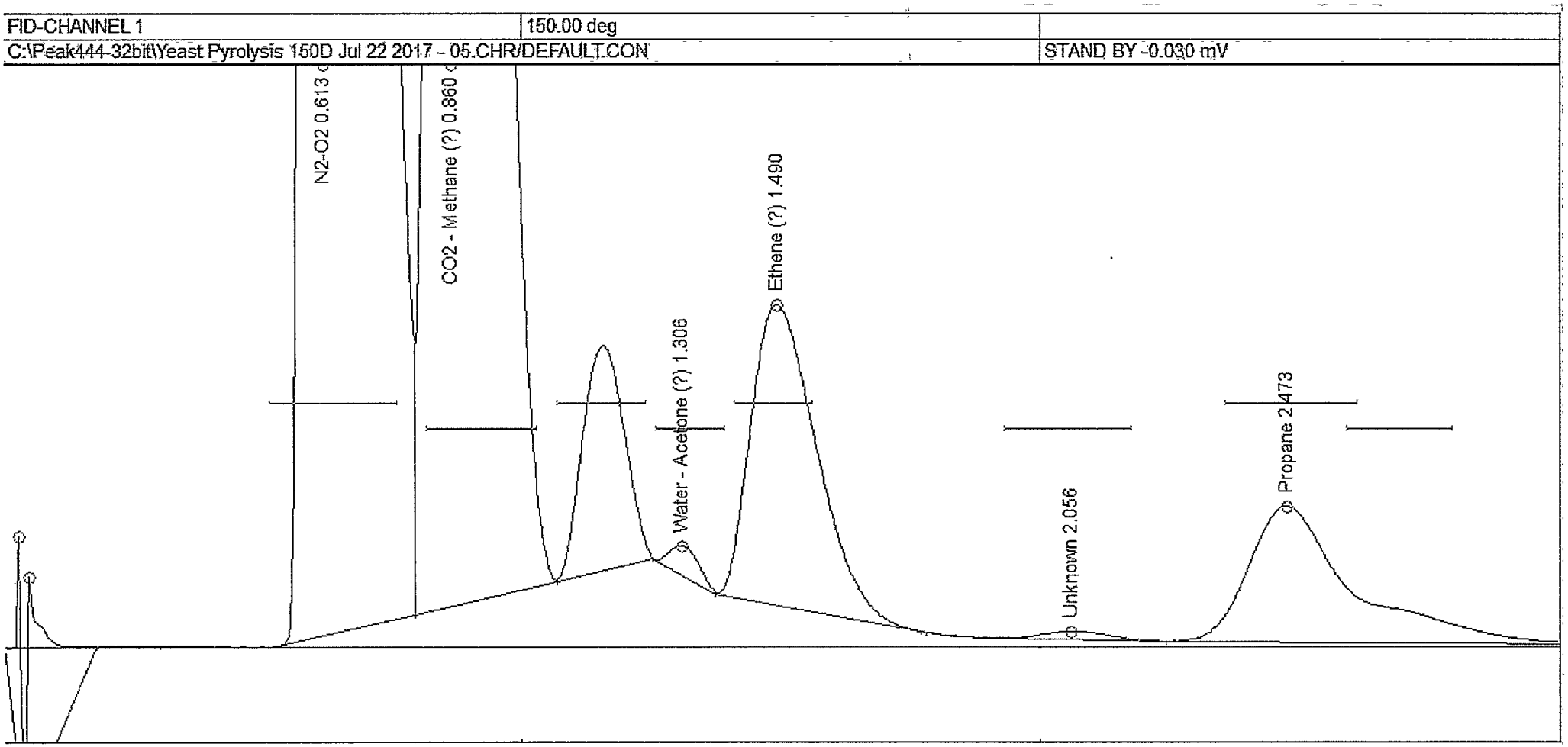
CDB Protein 150D Pyrolysis - Comparison with Dried Milk Jul 22 2017



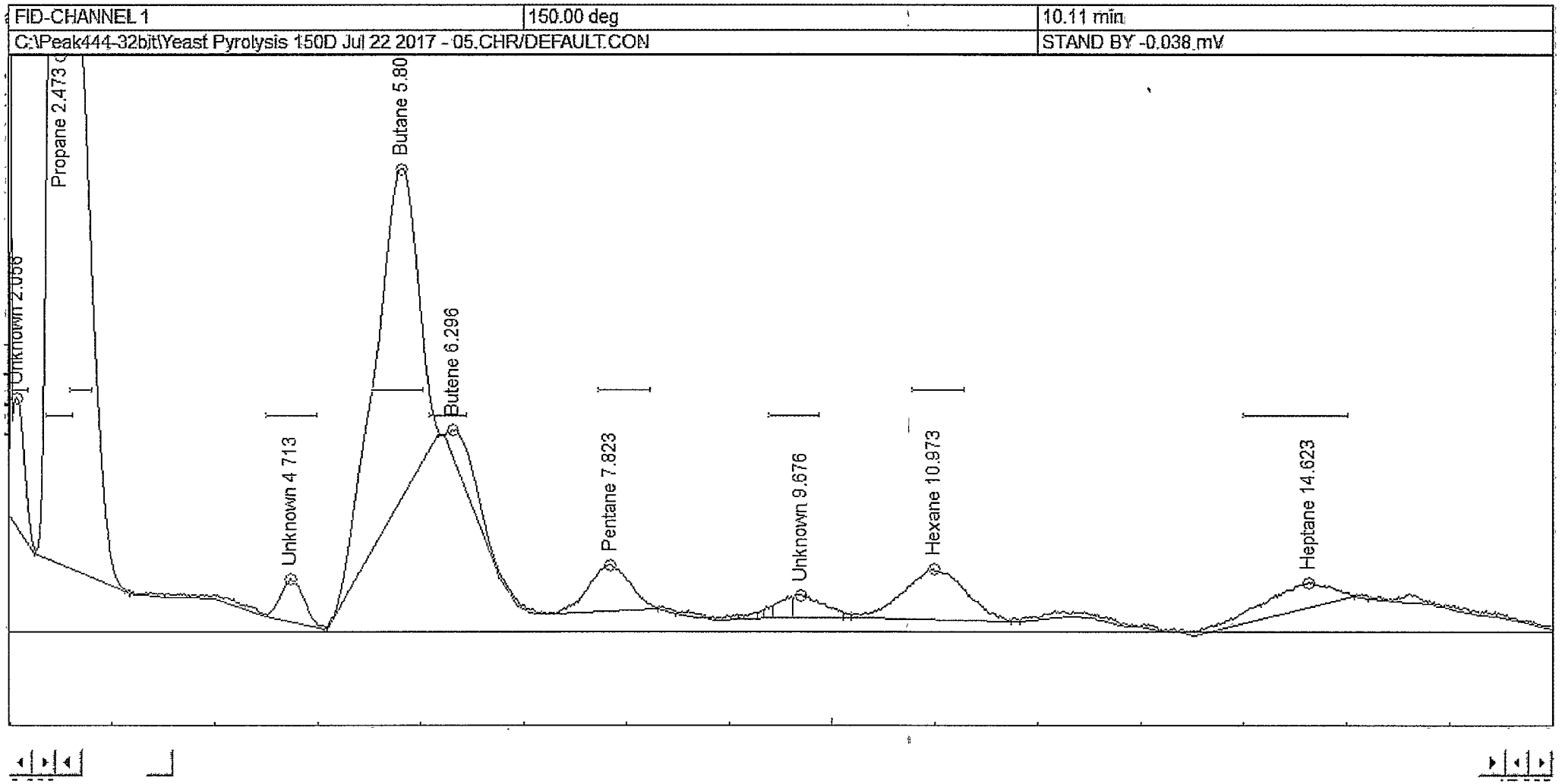
Page 220

Yeast Analysis - Hayes Sep D - 150°C

Yeast 150D Pyrolysis Jul 22 2017



Yeast 150D Pyrolysis Jul 22 2017



We already have C_{11} & C_{12} showing up on the CDB pattern pyrolysis.
After ramping to 220, we still have another.

We have 40 min @ 150.
Then we have approx 6 min @ ~210°C

this means 40 min @ 150 $\cong$ 20.65 @ 220°C
Then we add ~5 more min @ 220°C
$\cong$ 25.65 min

$CN \tilde{=} .73(25.65) + 1.5 \tilde{=} 20$

$MW \tilde{=} 10.24(25.65) + 23.7 \tilde{=} 286$

This gets us up to ~ C_{20}.
We even have another ~ 52 min @ ramp
So $t \tilde{=}$ 20.65 min + ~ 10 min = 30.65 min

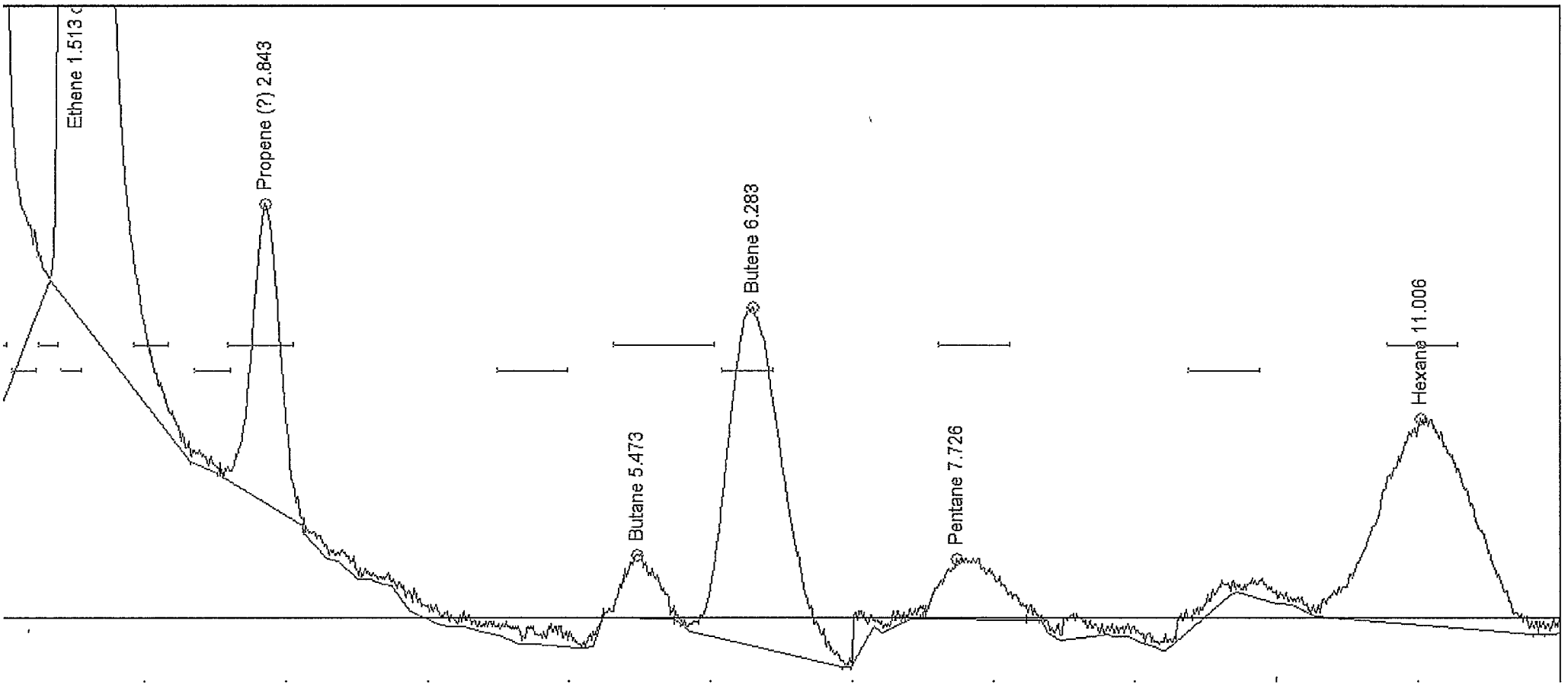
$CN \tilde{=} .73(30.65) + 1.5 = 24$
$MW = 10.24(30.65) + 23.7 = 338$
So now we have ~ C_{24}

So approx: $\Sigma \tilde{=} C_{100}$
C_{24}
C_{20} of saturated $C_{100}H_{202}$
C_{12}
C_{11} MW = 1402 min
C_6 (2) We have estimated ~4000
C_5
C_4 (2)
C_3 (2)
C_2

COB Secreted Hydrocarbon Production

Page 222 A

CDB Hydrocarbon Production 150D Jul 22 2017



Two short papers are due:

1. Protein signature (1st formalso?)

2. HC production.

Identical hydrocarbon production by the CDB from C_2 to C_6 has been verified by analyses of 2 separate cultures.

Tea Tree Oil Analysis - headspace vs pyrolysis - all show

methane	C_1	Tea Tree oil
ethane	C_2	~ C_{10}
ethene	C_2	
propane	C_3	so not had, either
		on the light
	$\Sigma = C_8$	track

You deduced that tea tree oil is a C_8 compound. Next you must determine molecular weight by freezing point depression.

Page 224

Charcoal powder from briquet lter:

N_2O_2 mix?

Methane	C_1	Ther seems like a pretty
Ethane	C_2	reasonable alternate to me.
Ethene	C_2	How to determine its
Propane	C_3	molecular mass? It
Unknown		will not dissolve easily
Butane	C_4	in anything.
Unknown		
Hexane	C_6	
	$\Sigma = C_{18}$	

From the candle method, we have identified CO as $t = 1.51$ on Hayes sep D @ 150°C.

CO_2 is @ 0.91
$O_2 - N_2$ is 0.60

You can really start to assess the C level in many compounds now.

Try Styrofoam again — $C_8H_8(n)$ MW = 104

Ethane	C_2	$C_{1.1}$?
Ethene	C_2	
Propane	C_3	
Unknown	3.95	$CN = 3.4$ $MW = 49$
Butane	C_4	(Data not seem like it should have shown up)

Call it a good day.

A small amount of contamination perhaps?