CARNICOM INSTITUTE LEGACY PROJECT

A Release of Internal Original Research Documents

Authored
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Dec 14 2018

Some items on the list:

1. Need to estimate MW of both proteins again since they have now been shown to be equivalent in the UV spectrum.
2. DNA extraction - getting closer
3. Then start project
3.1 The Mac Senior has arrived.

+ Explorations into potentiometry, open circuit potential (corrosion potential), conductometric titration, amperometric titration, potentiometric titration, are all just beginning. Redox reactions in general.

* Obviously the development of the protein involve significant redox reactions in the course of growth.

Activity series exploration - Tyler DeWitt on youtube education does a fantastic job of educating & communicating what is happening here.

I have an Open Circuit Potential (OCP) (corrosion potential) trial in place by the Palmeens. Curve is of the following form



w/a nail placed in the $CuSO_4$ solution.

Iron Nail in $CuSO_4$ solution:
Corrosion is a redox reaction

Half reactions (reducing) $Fe + CuSO_4$?

$Cu^{2+} + 2e^- \rightarrow Cu$ $+.337$ less active metal
$Fe^{2+} + 2e^- \rightarrow Fe$ $-.4402V$ more active metal

Fe is more active than Cu. Therefore it will replace Cu that is in solution.

So $Fe + CuSO_4 \rightarrow FeSO_4 + Cu$

$Fe^0 + Cu^{2+}SO_4^{2-} \rightarrow Fe^{+2}SO_4^{-2} + Cu^0$

Fe is oxidized. Cu is reduced. OILRIG

$Cu^{+2} + 2e^- \rightarrow Cu^0$ reduced $+.337$
$Fe^0 \rightarrow Fe^{2+} + 2e^-$ oxidized $+.440$
$SO_4^{2-} \rightarrow SO_4^{2-}$ spectator ion $E = .777$ V

Net:
$Cu^{+2} + 2e^- + Fe^0 + SO_4^{2-} \rightarrow Cu^0 + Fe^{2+} + 2e^- + SO_4^{2-}$

$Cu^{2+} + Fe^0 \rightarrow Cu^0 + Fe^{2+}$

Net Ionic Reaction

Etc
Cu is reduced
Fe is oxidized.

If we truly have an iron nail
We anticipate OCP to transition from $+.777V$ to $0.V$. Is this true?

Cu is formed (electroplating)
Fe^{2+} is now in ionic form.

Now for another round of molecular weight determination for the proteins (phosphate & non phosphate treated). Both forms of the soluble protein are now concentrated and purified more closely.

Brix values:

Non Phosphate version: 33.6
Phosphate version: 34.0
} Amazingly close - Good!

Non-phosphate version: Osmolality Data

(W_B/W_A)	mOSM
1/1	1282
1/2	828
1/3	635
1/4	524

We will use the slope regression method.

$mOSM = 992.68\left(\frac{W_B}{W_A}\right) + 300.23 \quad r^2 = .995$

$MW \cong \frac{IEG}{992.68} = \frac{1007}{} \text{ gms/mole}$

Previous notebook not accessible (in scanning) but the appear to be right in range with previous work.

Now for phosphate treated version

(W_B/W_A)	mosm
1/1	1411
1/2	925
1/3	701
1/4	561

$mosm = 1188.06\,(W_B/W_A) + 295.72 \qquad r^2 = .995$

$MW \cong \frac{1E6}{1188.06} = 842$

The mean of both proteins is: 925 gms/mole

I think that this is surprisingly close to our previous estimate.

We continue to maintain:

1. A water soluble iron based-sulfur protein (polypeptide) on the order of ~7 amino acids (not unique) along the chain.

Candidate amino acids also identified.

Run Comp

Non phosphate Protein 12/14/18

MULTI

Tech:

Date: Sam

Phosphate Protein 12/14/18

MULTI

Tech:

No need to remove during a can.

Another protein observation:
I suspect this will eventually become important as the study of electron transfer proceeds.

There are important color changes that take place in the protein:

If the protein is heated to ~ 70°C, it will turn a rich light green color. It is clear that what is happening is that the Fe^{+3} in the protein solution is being reduced to Fe^{+2}, ie ferric to ferrous.

In contrast, if the heated protein is allowed to set @ room temperature over the next several days in a capped container, two things will ensue:

1. A reddish layer will begin to form at the top of the protein surface.
2. As time continues, the Fe^{+2} will oxidize to Fe^{+3} and the protein will turn a rich brown color.

The process can be reversed by reheating the protein and it will once again turn temporarily to the light green rich color.

There is a reversible reaction involving heat and oxidation.

Dec 15 2018

Late last night, I have succeeded quite well with an open circuit potentiometry (OCP) titration trial.

I have used $FeSO_4$ & sodium dichromate. The Palmsens logging of data was especially helpful as well as the derivative function for the equivalence point.

I am developing a low power portable laboratory set up for field use:

1. Small stirrer (milk frother) battery-operated
2. 50 - 250 mL beakers
3. Stand w/ 2 clamps
4. Water meter: EC-pH-ORP tester

The plan is for some detailed investigations of electrochemistry during the water trip to Nicaragua

1. potentiometry.
2. electrochemical investigations of the soluble proteins.
3. titration
4. voltammetry & on w/ the Palmsens.

$FeSO_4$ (drops) & NaC_2O_4 (analyte)
Potentiometry Test w/ ORP & EC meter

EC	ORP	Drops
0.79	1030	0
.81	1080	1
.82	1090	2
.82	1060	5
.82	1010	15
.83	960	20
.83	910	25
.83	870	30
.84	825	35
.84	755	40
.83	680	45
.84	650	50
.84	640	55
.85	645	60
.85	685	65
.86	735	70
.88	775	75
.92	820	85

55 drops is the minimum.
55 (.06 mL) = 3.30 mL

Assume 100 mg analyte @ 1M

$M_1V_1 = M_2V_2$ $\quad M_2 = \frac{M_1V_1}{V_2} = 1\frac{(100)}{3.3} = 30.3\,M$

Example only
Smooth curve data.

Processing:
Based upon inspection, remove first two data points and the last point.
Cubic regression to still the best.

$ORP = 4.837E\text{-}3 drops^3 - .429 drops^2 + 1.182 drops + 1076.96$

$r^2 = .991$

Graphical minimum @ 58 drops
$58(.06) = 3.48 ml = V_2 \Rightarrow M_2 = 28.7 M$
Hypothetical molarity.

The method worked very well using ORP.

Drop method works well & is simple w/ equipment.

Equipment used only:
1. 25 ml beaker
2. EC & ORP sensors
3. Stirring rod
4. small pipette.

* Another observation: Acidified $FeSO_4$ keeps the Fe in the Fe^{+2} state.

It creates a perfectly clear solution.

Next reveal trial is
1. Blue food dye dilute - analyte
2. Dilute bleach - titrant

Initial ORP ≅ ~~850~~ 900

	Drops	ORP	
	1	980	
	2	1055	
	3	1085	
	5	1120	
	10	1135	Increases
	20	1140	
	40	1140	
	41	1145	Full Strength Bleach
	45	1150	
	55	1150	
	65		
add	1 ml	1170	
	3 ml		
	8 ml	1120	
	13 ml	1075	
	18 ml	Decreases 1044	Finally getting paler now
		1055	solution is clearing
		Increases 1065	solution almost clear
		1080	Solution is clear now

This was a very unexpected data set.

Dec 16 2018 Target Amino Acids of the Soluble Protein

A rather amazing observation.

The pH of the soluble protein (phosphate treated) is 3.2!! This is incredibly acidic. Previous estimates were on the order of 4.5, but now we have it even lower.

The protein under testing here is only moderately concentrated, estimate Brix @ ~ 15 or less.

This further confirms that highly acidic amino acids and polar are most likely to be involved here.

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* Hydrophilic, acidic amino acids are expected to play a dominant role within the protein

Targets:

acidic hydrophilic Aspartic Acid - used in the biosynthesis of proteins - precursor

acidic hydrophilic ~~Glutamic~~ Glutamic acid

Histidine - Hydrophilic

* Proline - Hydrophilic

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1. Company ORP vs OCP of identical solution would be valuable.

2. Sugar reagent (Benedict's) would be helpful in confirming the polarimeter

Dec 17 2018

Today I have made Benedict's reagent for the testing of sugar presence.

This will be especially valuable for monitoring the consumption of sugar within the cultures.

This will also be paralled w/ use of the polarimeter when an initial flood of Benedict's reagent.

As we have seen before Benedict's reagent is not especially sensitive. I think that there should be a much more sensitive test for sugar. Benedict is still very useful for more coarse detection.

Consider
1. UV
2. Polarimeter
] as alternatives if need be.

Important consideration w/ the use of Benedict's reagent.

Sucrose will NOT work w/ out hydrolyzation, ie, adding of HCl.

Sodium Carbonate hydrous requires more mass in Benedict's reagent than anhydrous.

Benedict's works w/ reducing sugars.

A reducing sugar is any sugar that is capable of acting as a reducing agent, because it has a free aldehyde or a free ketone group.

All monosaccharides are reducing sugars. Some of other sugars are

Sucrose is a non reducing sugar!

It does not have a free ketone or aldehyde group.

If you are going to try to work w/ sucrose, you are going to have to break it down into its monosaccharides: fructose & glucose

Also, make sure you use sufficient reagent. Enough to turn the solution blue from the copper, even if acid is added in an attempt to hydrolyze.

On the next page is recorded another important observation.

Reducing Sugar Present

Observation:

* We know that we positively have a REDUCING SUGAR w/in the soluble protein mature culture.

We had an extremely strong reaction (yellow precipitate - turbid) w/ 1 drop of the moderately concentrated protein (phosphate treated) w/ Benedict's reagent.

A very pronounced reaction upon heating. We do not yet know yet is a monosaccharide or not but glucose and fructose testing would be just on the agenda.

This is an important observation as it shows even additional microbe metabolic activity.

Additional observation: When the phosphate
* treated water soluble protein (of white or color) is placed in contact w/ white paper it oxidizes readily and causes a pronounced rust colored stain.

* Culture Production is Prolific & Complex

Benedict's colors:

green	~ 0.5%	sugar concentration
yellow	~ 1.0%	"
orange	~ 1.5%	"
red	~ 2.0%	"

We are in the yellow region therefore we anticipate a concentration of ~ ~~1~~ 1%.

Realize this was with a dilution ratio of 1 drop / 3ml

or 2-3ml / .06 ml ≅ 30-% 50 so we actually have an extremely high concentration level of the reducing sugar whatever type it is.

It is NO LONGER SUCROSE.

What we are learning here, therefore, is that our culture medium is prolific and producing:

* 1. a solid protein (high level of production)
* 2. a filament network
* 3. a water soluble, highly acidic protein (polypeptide)
* 4. a reducing sugar in high concentration

* This is a lot of metabolic activity from a single culture medium.

Dec 17 2018
Vit C titration experiment.
2.87 gms Vit C Crystals

Beaker weight 96.61 gms
W/ H_2O 178.30
Δ = 81.69

3.50 Weight boat
5.08 w/bleach
Δ = 1.58 gms bleach

Vit C : 2.87 gms
81.69 + 2.87 gms

= 3.39% Vit C

Glass Jar 190.00 ml
350.15 w/ H_2O
Δ = 160.15 H_2O
+ 1.58 bleach
161.73 gms

= 0.976% Bleach

Bleach = 1.58 gms
161.73 H_2O
+ bleach

Here is the first surprise to me.
Vit C is known to be a reductant.
I would expect a negative or low ORP reading. I have ~+350 mV to start w/ which is higher than expected.

I will notice if the value increases w/ a 0.976% bleach solution, which is expected.

Data: (30 second wait period) 1 drop = 0.042 ml

Drops	ORP	Assume 50 ml for example sake:
0	340	
5	348	
10	346	$M_1V_1 = M_2V_2$
30	342	
60	339	$M_2 = \frac{3.39\%(84.56)}{50\ ml}$
+1 ml	+336	
+3 ml	Increased bleach Conc. 332	
+5 ml	318	$M_2 \approx 5.73\%$
+5 ml	311	
+5 ml	full strength bleach 296	That's actually
+5 ml	285	reasonably well
+5 ml	273	balanced.
+5 ml	262	
+5 ml	256	Regular bleach is
+5 ml	257	5.25%.
+10 ml	252	Look like it might
+10 ml	here it is 362	be on the right track
+10 ml	496	here

So we did indeed have an equivalence point @ ~ 70 ml ⇒ ~ 50 ml full strength bleach.

It took a surprising amount of bleach to find the rate increase.

Notice a gradual descent.

Trial 2: 30 sec delay in reading ORP.
VHC = 2.93 gms full strength bleach in used.
H_2O : 89.44 ml
% = 2.93/(89.44 + 2.93) = 3.17%
Total 92.24 mean

	Drops (or ml)	ORP mV		Drops (ml)	ORP
sec	0	415	00	61	336
Σ	10 ml	384	00	62	338
Σ	20 ml	361	00	63	340
Σ	30 ml	346	00	64	342
Σ	35 ml	339	50	65	344
Σ	40	339	45	66	347
Σ	42	333	30	67	349
Σ	44	338	20	68	354
	45	335	05	69	357 Δ
	46	335	50	70 on its way.	366
	47	334	55	71	436 70
10	48	336	45	72	531 95
05	49	334	35	73	531
00	50	333			
00	51	336			
00	52	333			
00	53	333			
50	54	336			
10	55	333			
00	56	334			
55	57	333			
50	58	335			
45	59	335			
30	60	335			

$\bar{X} = 71.5$ ml

$M_1 V_1 = M_2 V_2$

$M_2 = \frac{M_1 V_1}{V_2} = \frac{3.17\% (92.37 ml)}{71.5 ml}$

= 4.095%

These appear to be very solid results.

The theoretical concentration is 5.25%, however I have high confidence in the data set and brands may well vary, especially from the Dollar Store.

The method works quite well with a minimum equipment.

1. Ring Stand w/ 2 clamps
2. ORP / EC / pH meter
3. 250 ml beaker
4. Analyte in beaker (in this case a Vit C solution) of known concentration. (or visa versa).
5. Titrant in a reservoir
6. Two syringes (60 ml & 10 ml) for rough & fine titrations also drop calculated
7. Stirring Rod.

Perform rough titration first, then fine titration. No power required for the method.

Note: A digital instrument is expensive but offers some advantages. Volume / drop counter, automatic stirrer, Calculations ORP vs pH probe vs EC is an issue!

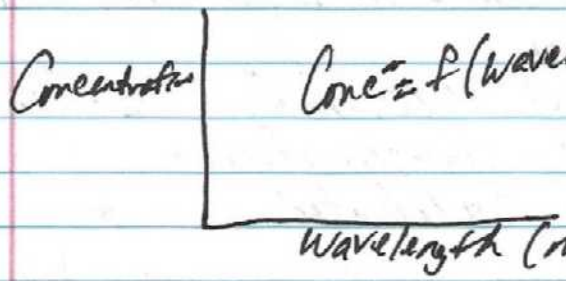
The method is very inexpensive but tedious. Automation certainly is valuable here, but pH probe is limited in application.

It looks to me like Benedict's reagent results can be applied to VIS spectrometry and concentration models developed.

The precipitate does settle out but the color of solution does remain.

This could be done by centrifugation.

I anticipate a model of



since the color varies w/ concentration.

But do all sugars behave the same or will need a calibration for each type of sugar?

Dec 18 2018

Now that we have a basic & relatively low tech method of titration in place (ORP), now let's go back to the Palmiera to attempt at least a partial logging of data collection.

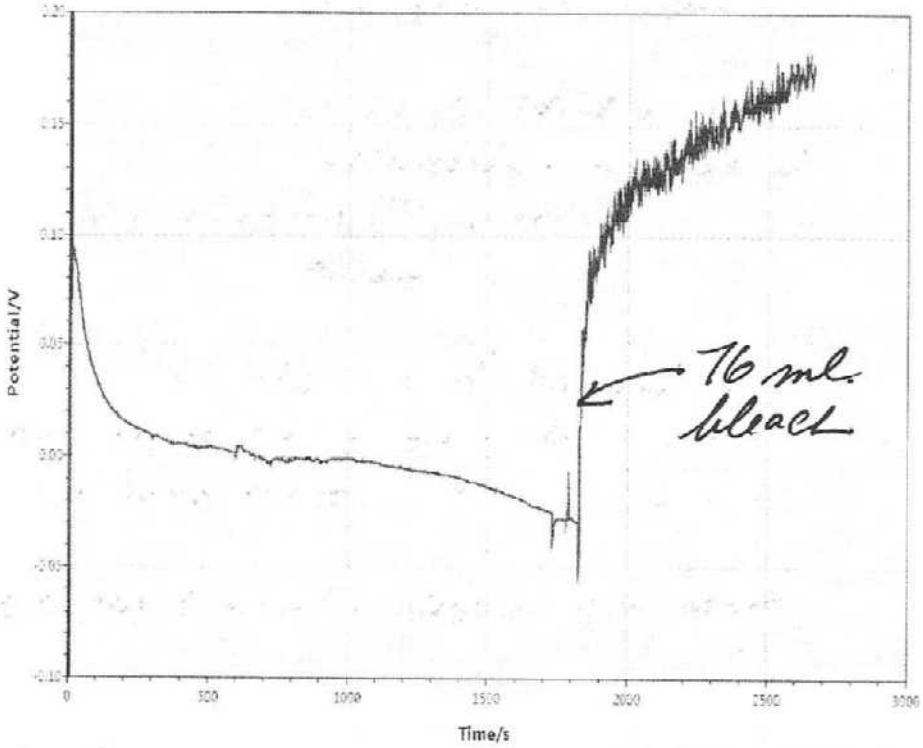
3.01 gms Vit C %= 2.69%
111.86 Vit C + H_2O

Well attempt to log the voltage; automatic Stirring graphite electrodes (working & reference only)

Bleach		V	
	0	.004	after settling for 10 min.
	25 ml	.000	delay = 1 min / 30 sec
0	35 ml	−.001	Bleach Vol
15	40 ml	−.001	45 72 −.028 w/spike
45	42	−.001	30 74 −.028 w/spike
0	44	−.001	15 76 +.076 SPIKE
15	46	−.002	Voltage Continue to rise.
10	48	−.004	Now @ 0.134 V, Next @ .175 V
10	50	−.005	Slowly stabilizing.
15	52	−.006	The + our point
10	54	−.007	
10	56	−.008	$M_2 = 2.69\%\left(\frac{111.86}{76\ ml}\right)ml = 3.96\%$
0	58	−.010	
45	60	−.012	vs 4.095%
50	62	−.015	$\bar{x} = 4.03\%$
30	64	−.017	
15	66	−.019	Excellent results.
00	68	−.022	
45	70	−.024	

Titration Curve - OCP
Vit C - Bleach

Date: 18 December 2018



The method of titration w/ the Palmsens using Open Circuit Potential method was superb.

You have excellent data now by two methods: manual & partially automatic.

The Palmsens method will log the data.
Only manual operation is drop count or
syringe delivery.

Equipment required:

1. Ring stand w/2 clamps
* 2. Palmsens software & computer
3. 250 ml beaker (analyte)
4. Titrant in a reservoir
w/ 60 ml & 10 ml syringes
drop calibrated
* 5. Battery operated stirrer (modified milk frother)
a small Dremel tool
* 6. Graphite Electrodes (double sharpened pencils)

* = Differences from the manual method.

Palmsens is far preferred as we get graphical logging of the data.

The Palmsens method may negate the need for a stand alone titrator.

There was superb work.

Next: DNA

The DNA extraction project has started. It has been about 1½ years since the last project - which was successful.

I will need to start up again from previous notes and build up the protocol again.

If I succeed it will be the third time I have done this. One difference is that I now have UV capability which should help in the verification process.

I am starting, once again, with banana as a test case, since it is one of the easier examples to work with.

I have succeeded w/ it, but it was not exactly straightforward.

The trials have been run, each successive one performing better. The first trial can be rated as a failure, the second w/ very mild but observable success, and the third w/ moderate success.

Time may also be a factor in the success, esp w.r.t. potential enzyme activity.

The base recipe is:

1. 100 ml sample (including liquid)
2. 4 ml enzyme detergent (oxclean)
3. 3 gms salt
4. Two full microscoops of multi-enzyme
5. Pulse sample (the time estimate for the remain uncertain) time of setting in blender is also a factor)
6. Straining and cooling down of sample are also variable.
7. Cold denatured alcohol (not isopropyl - no water)

Fourth trial is also successful

Notes:

1. Dice up sample small enough so that it is easy for the blender to handle it.
2. I only pulsed sufficient to liquify the sample.
3. I did use strained sample material
4. The alcohol is now setting colder.
5. The sample did not need to be cooled down
6. Whether the pureed sample improves by setting over time is still unclear.

Form heater sample w/ the semi solid materials:

I have fantastic success using the blender method.

1. Approx 20 ml of the pureed sample (pulsing was minimal in the small blender)
2. Approx 25 ml of chilled denatured alcohol

Massive production of DNA.

Infrared spectrum taken on opposite page.

Below microscope image & UV spectrum.

—

Well, I have to say it is more than interesting that our best match in the IR database is actually the CDB secreted protein sample of Nov 27 2017 $r = 0.932$

That is certainly a question and predicament to work out, isn't it?

I found the data to construct the regression on Wikipedia.

Two different methods:

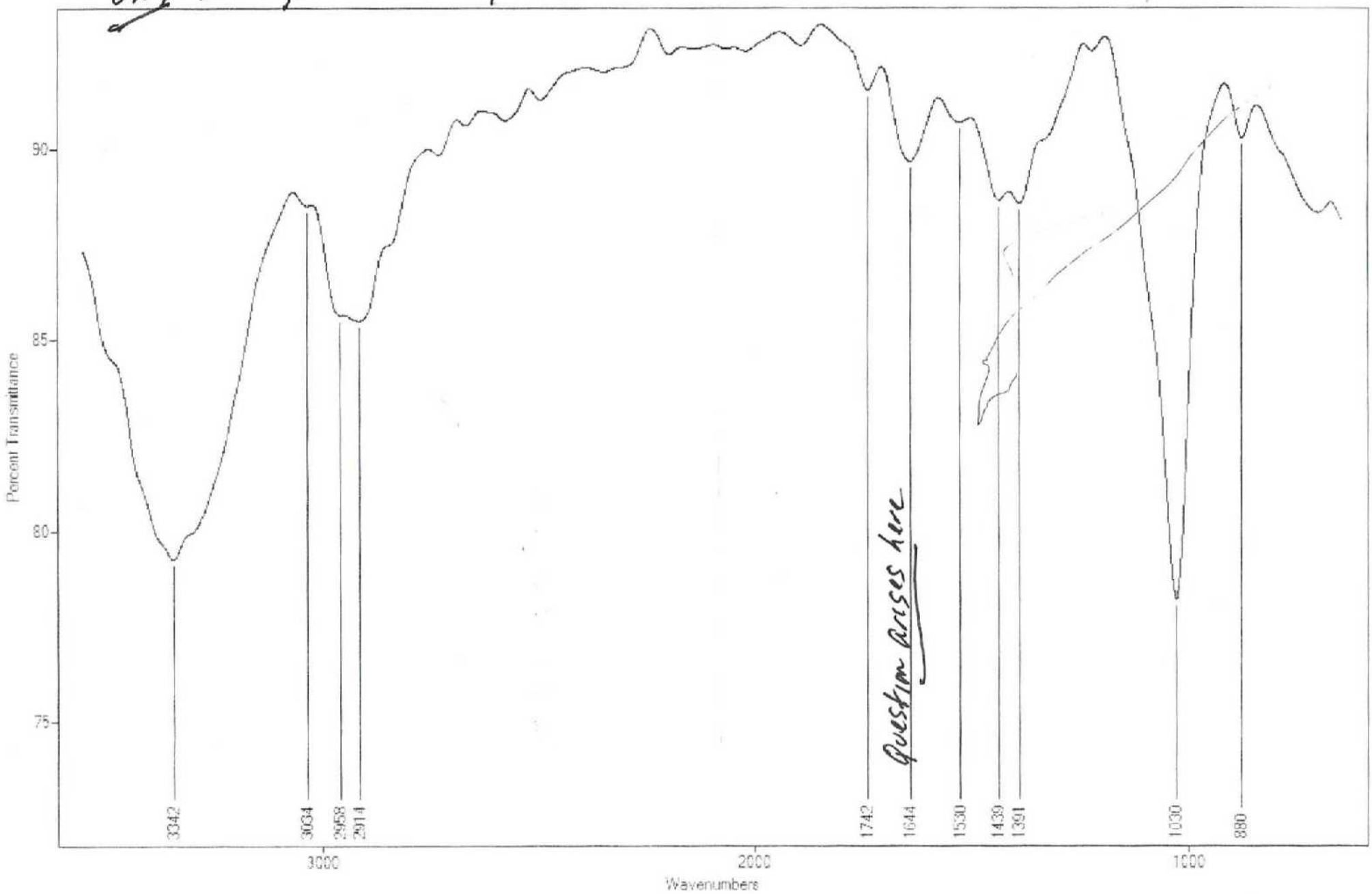
Primary signal here is the denatured alcohol
Negative – see notes for Dec 19 2018.
Reference DNA – Banana – IR Spectrum



See Notes Dec 19

DNA Banana ATR Dec 18 2018 - 01.spc: Synthetic spectrum by averaging. See audit trail for details.

Only 1644 peak is of high interest here.



printed on 12/18/2018 18:41:33

% nucleic acid	260/280 ratio
0	.54
5	1.06
10	~~1.06~~ 1.32
30	1.73
100	1.8

Our ratio is currently 1.16 w/ the linear sample.
Not @ all linear!

We need to use just the first three data points.

% nucleic acid = 12.93 (260/280) − 7.71 $r^2 = 0.97$

% = 12.93 (1.16) − 7.71 = 7.3 %

Ok, this is actually a very usable number
even if it sounds low.

I also think we have an alcohol influence in the absorption.

An alternate regression is:

% nucleic acid	260/230 ratio
100	2.00
95	1.99
90	1.98
70	1.94

Our 260/230 ratio is .0317

We can not do this. Our alcohol is very
high in the 230 range.

We do indeed have high alcohol absorbance in the region.

Running a trial of 1 drop of alcohol in 3 ml cuvette of H_2O

Ok, it is indeed very high.

λ	A
396	.0365
306	.0554
280	.0980
260	.1262

A scaled version of the absorbance curve needs to be subtracted from our DNA spectrum.

A power regression is sufficient for now.

$A = 1.236(\lambda)^{-2.913}$ E6 $\quad r^2 = .91$

Our DNA absorbance @ $\lambda = 396$ $= .0041$

Therefore we need to scale our regression by a factor of $\frac{.0041}{.0365} = .112$

Therefore our Absorbance Regression spectrum to be subtracted is Reference

$A^* = (.112)\left[1.236E6(\lambda)^{-2.913}\right]$ at 260 & 280 nm

$A^*260 = .112\left[1.236E6(\lambda_{260})^{-2.913}\right] = .013$ subtract!

$A^*280 = .112\left[1.23E6(\lambda_{280})^{-2.913}\right] = .010$ subtract

Therefore the adjusted value of the DNA spectrum are

$260 - A^*_{260} = .0316 - .013 = .019$

$280 - A^*_{280} = .0270 - .010 = .017$

and $\frac{.019}{.017} = 1.12$ essentially the

same as before even after correcting for the potential alcohol in the sample

We will hold to an estimate of 7.3% purity of the banana sample DNA for now.

The means we would like to collect as much of the DNA from the COB as possible.

It does seem strange that the purity is estimated the low considering you appear to see pure DNA strands in the alcohol separation process.

The most favorable UV plots are obtained just after the denatured alcohol evaporates but before it dries completely. This gives the strongest signal. Remember that there is no water present.

We do have the DNA.

IR Plots of DNA Compared: CDB vs Banana

If the sample dries entirely you lose the signal

Major Correction and revision here.

See note for Dec 19 2018

Because the protein (solid) generated by recent culture methods is "soft", or less dense than the CDB, it has been much easier, it appears, to extract the RNA DNA under these conditions.

IR Plots match to a very high level.

OK, backing up now.

The UV plot of the CDB beaker (large scale) purported DNA does not match that of the Banana DNA UV spectrum. This casts doubt on the veracity. It may be highly protein contaminated.

We have two small test tube samples that strongly demonstrate the behavior of isolated DNA. The samples are very small and therefore valuable.

I think that I will run additional test tube method sample (filtered) before dissolving the a small sample in H_2O where it is no longer viable.

I am after a general UV match of the banana sample with higher absorbance in the 260 nm region.

We have no CDB DNA transfer into alcohol (or formation) from the puree sample that has been sitting for the last two hours.

We must now start w/ a fresh trial. It may be that only very small sample can be extracted.

10 pulses used in the small blender.

Hold your hands over the Buchner funnel when vacuum filtering the puree into the Buchner flask. This is much more effective at drawing out all the liquid from the puree.

I managed to get 5 test tube samples this way ~~so~~ instead of the former two. (~5 x 5 ml ≈ 25 ml)

Sample size of CDB concentrate solid protein is ~30 ml.

This time I ~~to~~ cut the salt and soap samples in half (approx) because the original sample is only 30 ml instead of 100 ml.

I see very light bubble formation taking place. We well let them ~~at~~ sit about 15 min.

Two of the 5 tubes are slowly forming DNA. Another tube has a trace of formation. Two tubes are failing completely.

This is difficult, but I am getting there.

DNA samples will be small & precious from the CDB plotein but it will be possible.

Suspect about 10 trials will be required to produce a usable amount for a laboratory.

Although small, the formations do look clear and pure, as in the original 2 test tubes.

The heater method appears highly suspect in the case.

You will need to repeat these trials carefully.

Main variables now are
1. No of pulses in blending puree
2. Salt, soap & enzyme combinations.

Your filtering of the puree via vacuum is now very efficient and the filtered sample & DNA extractions are visibly clean looking.

The two samples that are producing DNA are getting ready to separate and float to the top.

I am going to repeat the banana DNA a

a reference also and I will also use the
test tube method.

Dec 19 2018

Next trial. I will use 50 pulses instead of 10.
3ml soap, 2gm salt.

Also, let the puree set for about 10 min before straining.

—

This trial w/ 50 pulses did not work @ all.

—

Let's try 5 pulses w/ no wait.
3ml soap
3 gms salt
2 enzymes

I acquired a small amount in the beaker approach.

—

This is difficult, no way around it. But I do have enough now for a laboratory to analyze.

I am also shifting to the beaker method as I think that an increased surface area - alcohol interface increases the likelihood of production. I notice in our last isolated samples w/ floating DNA that there is solid material that has settled. I believe this is only contamination of the sample.

DNA IR Notes - Revisions

I would like to isolate enough material to, as an extra, record it under the microscope as well as IR.

This will be demanding, but should be possible.

—

A major observation w/ the attempted DNA IR plots.

For the time being, they are to be almost entirely disregarded.

The signal you are picking up in the IR plot is the Denatured Alcohol, it is not DNA absorption.

We do not have reliable or useful DNA IR information @ this time.

This is why the signal "disappeared" when the sample was known to be entirely dry. There was no significant absorption.

The only distinction I can currently make is @ 1654 cm⁻¹ the denatured alcohol did not have absorption here. The DNA sample may.

I will look @ the max closely from now on.

The literature does indeed show absorption in IR for DNA

however we may need to dissolve the sample in H_2O & evaporate that.

Sample prep might be an important issue here.

—

I am finally getting a handle on sample prep for DNA / IR. It must be dissolved in H_2O and then evaporated as a film on the ATR.

We have our first decent DNA IR plot on the next page. It no longer has any association w/ denatured alcohol. Very helpful here as a base reference

—

This also improves the UV spectrum collection as it reduces the denatured alcohol interference.

For the Banana dissolved DNA reference:
Our 260/280 ratio is now 1.19 ⇒ 7.1% DNA
We seem to have a usable sample

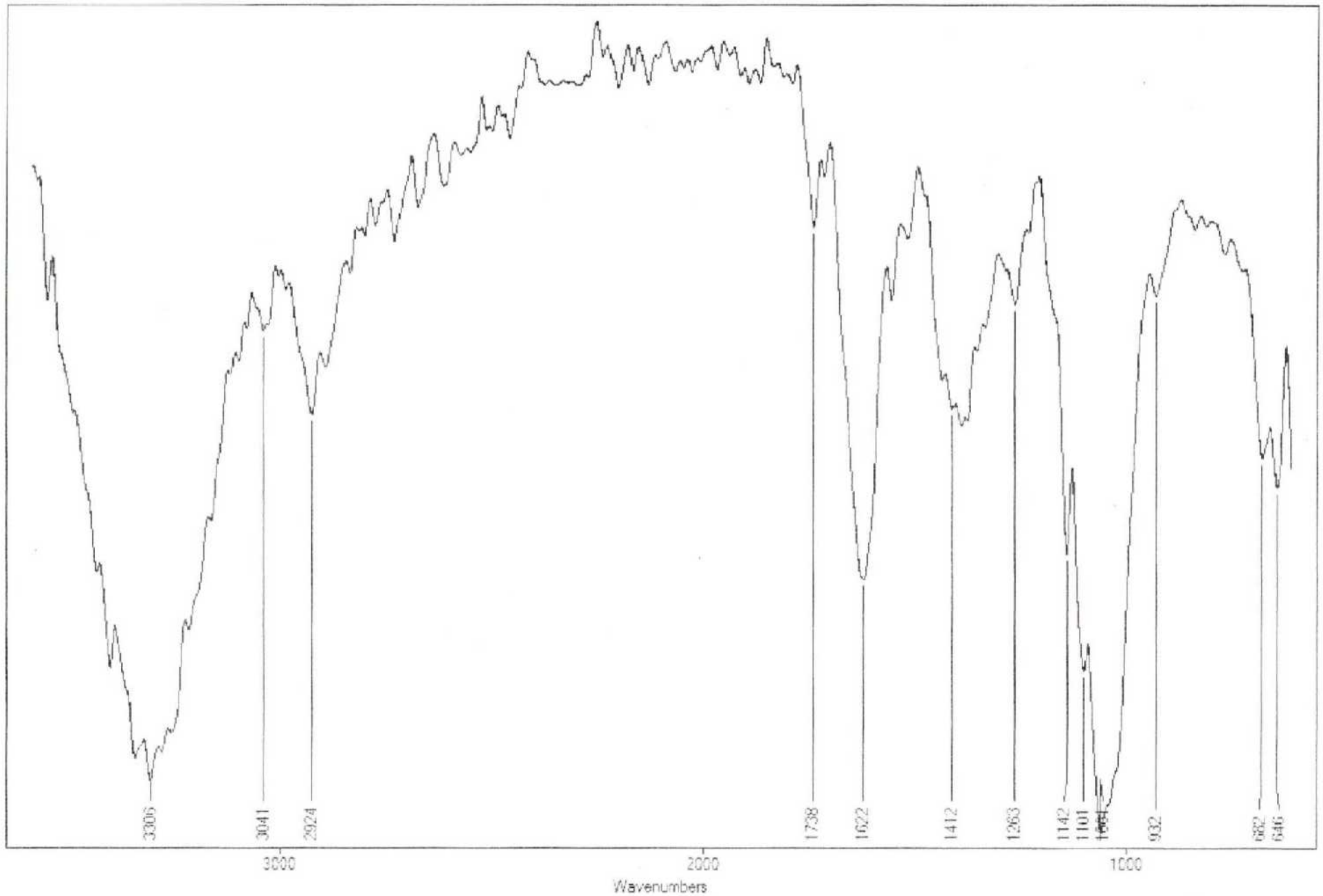
Viable Reference DNA IR Spectrum

Reference DNA - Banana - Dissolved in H_2O, Evaporated.

DNA Reference



DNA Reference Banana - Purest Sample - Partially Smoothed - Dec 19 2018 - 02.spc: 12/19/2018 15:30:38 title



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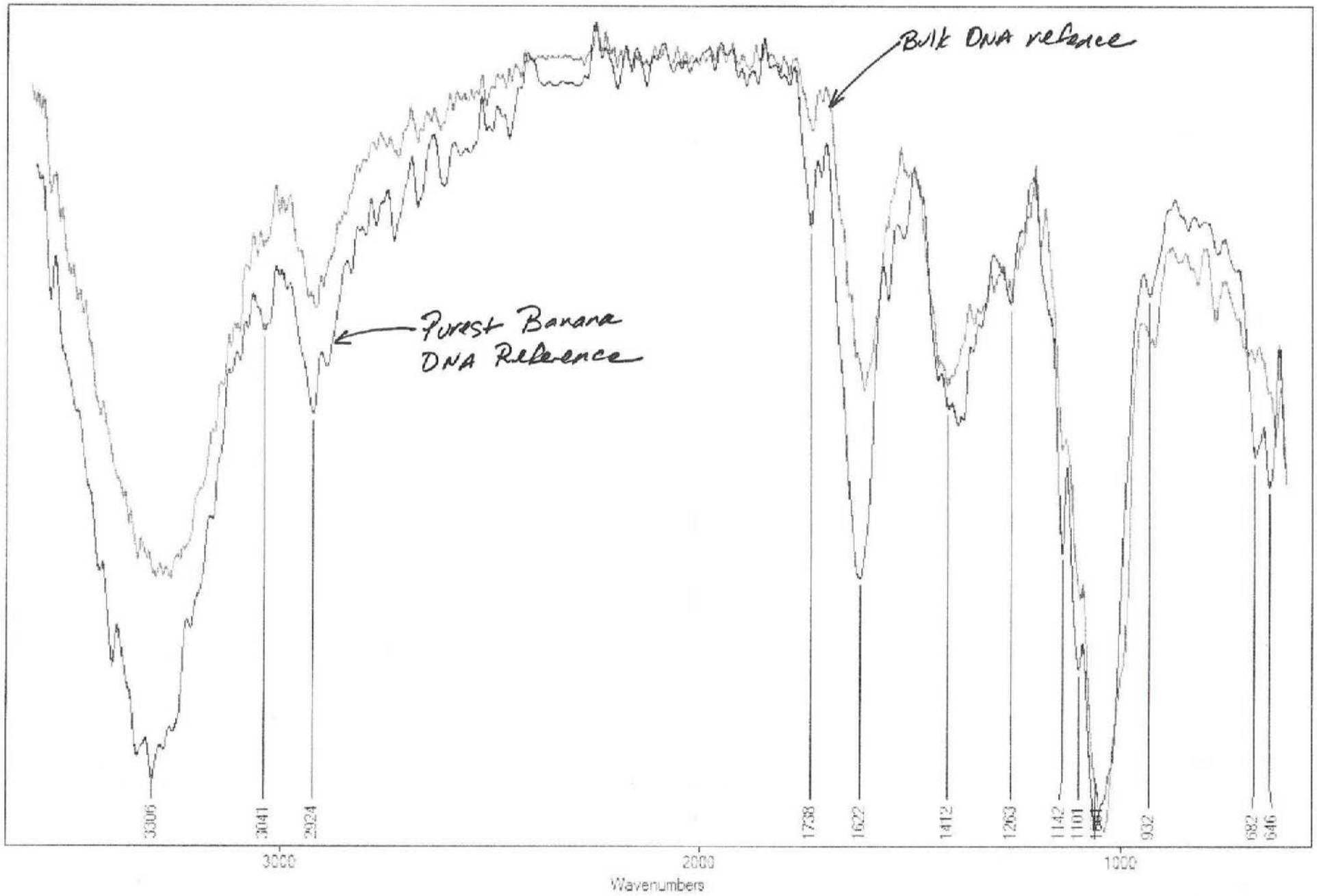
DNA Reference Banana - Purest Sample - Partially Smoothed - Dec 19 2018 - 02.spc: 12/19/2018 15:30:38 title



eFTIR

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We also know, now, based upon the excellent banana DNA reference spectrum, that the bulk, coarse spun attempted collection of DNA was a failure.

The sample is to be disregarded. It may be a protein related that is insoluble in denatured alcohol but soluble in water, but that particular sample is NOT DNA.

The means our collection of DNA from the CDB is small and difficult, but we believe we have a viable sample.

When enough material is collected it can be tested w/ IR & Compared to our reference spectrum.

The latest run has been quite successful. Went back to original formula

1. ~30 ml sample - 40 ml CDB Protein (Solid)
2. ~4 ml Oxy clean detergent
3. 3gm NaCl
4. 2 scoops Enzymes

Blended continuously the time for about 10 secs, until liquid looked uniform.

Verification of CDB DNA Extraction is in Place

Drained the pellet in 3 stages. It seemed to continue to improve w/ each drain / pellet.

I used the vacuum pump for the second run & with the passage of either time / concentration we may have improved.

The third run I packed the pellet with 2 more pellets and squeezed the remainder for another tube.

On alcohol, I added it fairly quickly, I washed one tube before going on to the next.

We must also realize that our solution may contain some residual detergent, enzyme, salt but it is minor relative to the extracted DNA.

Ok, we have a much weaker signal w/ the CDB DNA but we have it. There is no surprise based upon the mass of banana DNA that can be produced & placed in solution.

The correlation in the IR spectrum between the Banana & the CDB DNA is, however, undeniable. We do have DNA now successfully extracted from the CDB.

Verified by the chemical extraction process & by IR spectrometry.

IR

Comparison of Reference (Banana) & CDB DNA

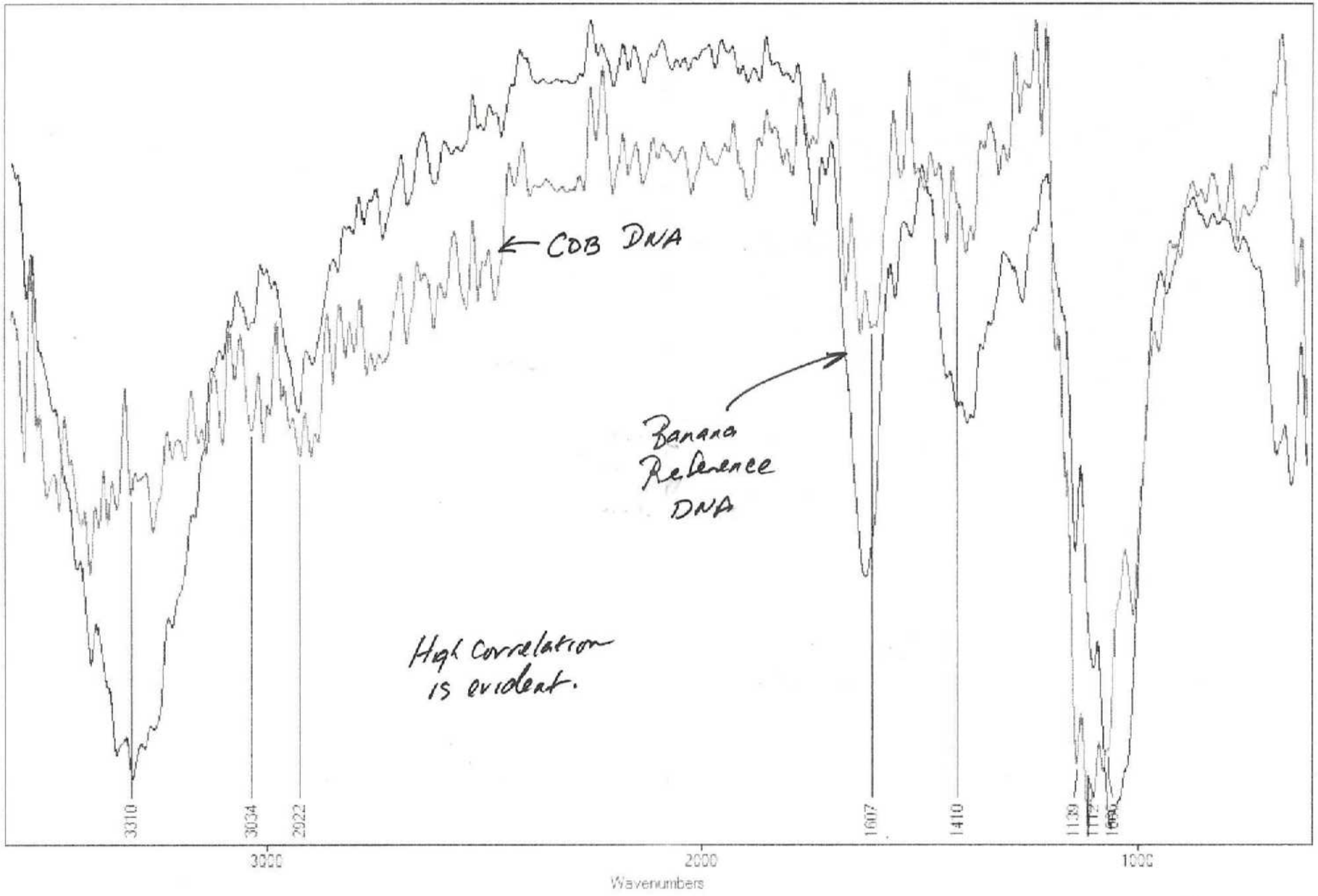
High Correlation exists.

CDB Spectrum has a much weaker signal

DNA

printed

DNA CDB Lightly Smoothed Avg Dec 19 2018 - 02.spc: Synthetic spectrum by averaging. See audit trail for details.



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Titration: Blue Food Color Dye + Bleach -30 sec delay

ml + drop		mV	Color
0 ml		-8	Rich Blue
2 ml		+50	Lighter Blue spiked up to +76 mV then descends and tapers to ~ +45 mV
148	10d	+46	light blue
0	20	+45	
0	30	+44	
0	40	39	
0	50	36	
0	60	34	lighter blue
15	70	30	
15	80	30	
15	90	29	
20	100	27	
20	110	26	
15	120	24	very light blue
15	130	23	
15	140	22	
30	150	22	clear
		31	
		35	

$\frac{1900 \text{ drops}}{7.95 \text{ ml}} = \frac{1}{X}$ $X = .053 \frac{\text{ml}}{\text{drop}}$

t = 2000 sec

t = 2500 sec gradual steady increase

t = 2800 sec

Let's go back a repeat three step rule.

60 ml syringe : 0.042 ml/drop

Note: 10 ml syringe : 0.053 ml/drop

Titration: Blue Dye vs Hypochlorite

Σ Drops or ml		mV	t (s)	Notes
	0 drops	+18	100	Deep rich blue
55	5	36	180	Lighter shade detected
		50	300	
		54	600s	peaked, normal blue
		58	850s	Stabilized, normal blue
30	6	59		
30	10	61		
30	15	63		
20	20	64		
0	25	65	1150	lighter blue
0	35	66		
20	50	68		
15	60	69		
30	80	71		
→ 30	100	72	1450	light blue
30	120	73		
45	150	72		
15	170	73		lighter blue
30	190	73		
0	230	72	1860	
30	270	71		almost clear

Peak reached @ ~ 100 drops ≈ 5.3 ml

Assume 95 ml of Blue dye assume 4% Bleach

$M_1V_1 = M_2V_2$ ~~$M_1 100 = 95$ ml~~ $M_1\ 95\text{ml} = 5.3\text{ml} * 4\%$

$\frac{4\%}{.22\%} = 18\times$

$M_1 = \frac{5.3(4\%)}{95} \approx 0.22\%$ Blue dye

≈ 0.21 gms dye ??

Possible

Dec 20 2018

I have improved the stirrer mechanism for titration w/ a battery operated milk frother with the top cut off. I now have a battery operated, quiet, mild stirring apparatus. The only real questions are how long the batteries last & durability.

—

I have started another trial w/ blue dye & bleach. As has been learned and is suspected, a single drop of bleach is sufficient to start the OCP change.

There has been a rise from 0.0 mV to +31 mV in 2000 sec w/ a single drop of bleach. Only a very mild color change to a less deep blue. It is leveling off @ +31 mV @ 2100 s. Add another drop of bleach.

The time the bleach has been diluted by about 1/3.

The plot shows that we are dealing w/ an immediate reaction here, not a redox reaction w/ an equivalency point.

The reaction mechanism is described in:
"Everyday Investigations for General Chemistry"
by Sally Solomon. Experiment 20.

Incidentally, it IS listed as an oxidation reaction.

Blue Dye ~ Diluted Bleach Titration

drops	t	mV	
0		0.0	Deep rich blue
1		+31	
2	2760	+34	
6	2950	+36	Strong blue
16	3050	+35	
66	3270	+28	Blue
	3400	+28	
1	1000	+24	Extra data point
+ Aml	3500	+22	Lighter blue
	3600	+22	
	3600 + 100	+22	Continuing to lighten
+ 4ml	+ 400	+21 mV	Light blue
	+ 1005	+20 mV	Clear

Stable pH + clear color

Absorbance will provide different data than potential does. Potential has already reached a max @ t ≈ 2500 sec. Color continues to decay gradually. Differing reaction mechanisms are being described here.

Stability in both process, however (color and potential) does merge @ t ≈ 4300 secs

@ Carrots Bleach Titration (Dilute Bleach – Dilute Carrot Juice)
Two additional reducers are supposedly Carrots & Mg.
I have made carrot juice.

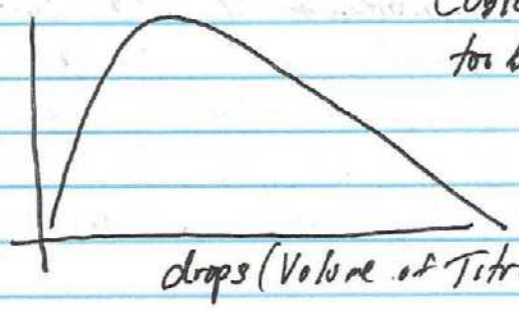
t(s)	drops	mV	Notes
1034s	0	0.00	Orange color-Carrot juice
	1	"	
	5	"	
1420	15	+3	lighter orange
1600	25	+10	lighter still
2100	35	+11	paler still
2400	60	+9	very weak orange tinge
3000	110	2	clear

Cubic regression is definitely the best and most realistic.
There are at least two major advantages of the drop-syringe method:

1. You can start and stop the titrant input @ any time to follow through on the extent of the reaction prior to continuous injection

2. You can change the magnitude of injection at will on an order level. Therefore titrant injection is very flexible depending upon observation of the reaction.

Cubic regression is not too bad, $r^2 = .903$

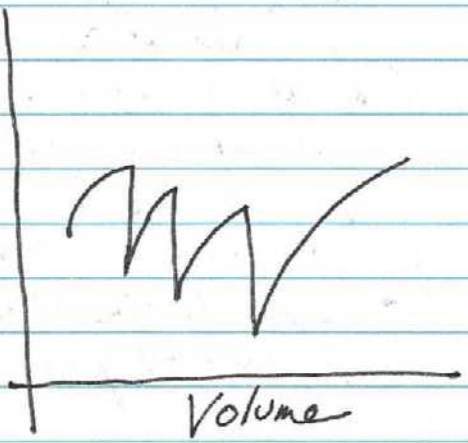


The titration is an example where the color loss does coincide w/ the stabilization of the reaction.

Peak potential, however is skewed toward the earlier portion of the reaction.

Each OCP curve appears likely to have some level of uniqueness attached to it.
Now try a stronger solution of the carrot juice w/ full strength bleach. (~25 ml Carrot juice.

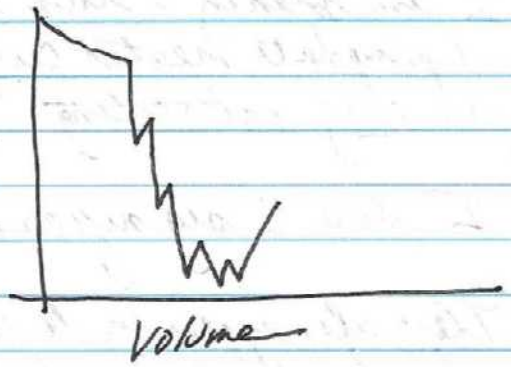
t	Vol (drops)	mV	Notes
525	0	+20	Stabilized
	1	+19	
	5	20	
	10	20	
	15	20	
	25	19	slightly paler
Sharp spike down to -3 mV →	1350	40	~18 spike down to -3 mV
sharp spike down to -1 mV	2380	60	~20 weaker orange
sharp spike down to +5 mV	3600+ 1850	90	44 pale orange



The interpretation of this OCP is interesting We assume increase in mV are oxidation and decrease are reduction. We have sharp reductions (increasing amounts of bleach - notice) and more gradual but escalating increases. This would make sense w/ the gradually increased oxidation of the sample but it also indicates the reductive capacity is strong and short-lived.

The time we will add 2 drop every min. Read on odd minute
Dispense on even.

Reaction: t	vol	mV	notes	t	vol	mV	notes
150	0	+45 1					
2	2	44 3					
4	4	43 5					
	6 6	42 7					
	0 8	41 9					
Change to 4 drops	10 12	40 11					
Change to 5 drops	12 17	39 13					
Δ=10	14 27	26 15	sharp spike				
	16 37	21 17					
Δ=20 (1224)	18 57	15 19					
Δ 30	20 87	10 21					
Δ=50	22 137	11 23					
	24 187	14 25					
	26 237	17 27					
	28	29					



This is a much better approach.
Steady dispensing.
Alternate dispensing & reading
data every other minute.
Flow rate adjustable as required.

We can see that once again a cubic is a very suitable regression. $r^2 = 0.982$

Steepest slope occurs @ ~ 40 drops, +22 mV = 2.1 ml
Min occurs @ ~ 110 drops, +8 mV = 5.8 ml

Assume initial vol. of carrot juice is 26.4 ml
$M_1V_1 = M_2V_2$ $M_1(26.4\text{ml}) = (4\%)(5.8\text{ml}) \Rightarrow M_2 = 0.9\%$

Ratio is ~ 4 to 1 in terms of oxidizing capacity.

Dec 22 2018

We have some very significant Liquid Chromatography taking place today. (LC)

I am making a run with the soluble protein (phosphate treated culture) and the results are very interesting.

I have 3 significant separations already.

There also appear to be up to three more separations by band color, and one of them may even be a purple color.

My separations currently are

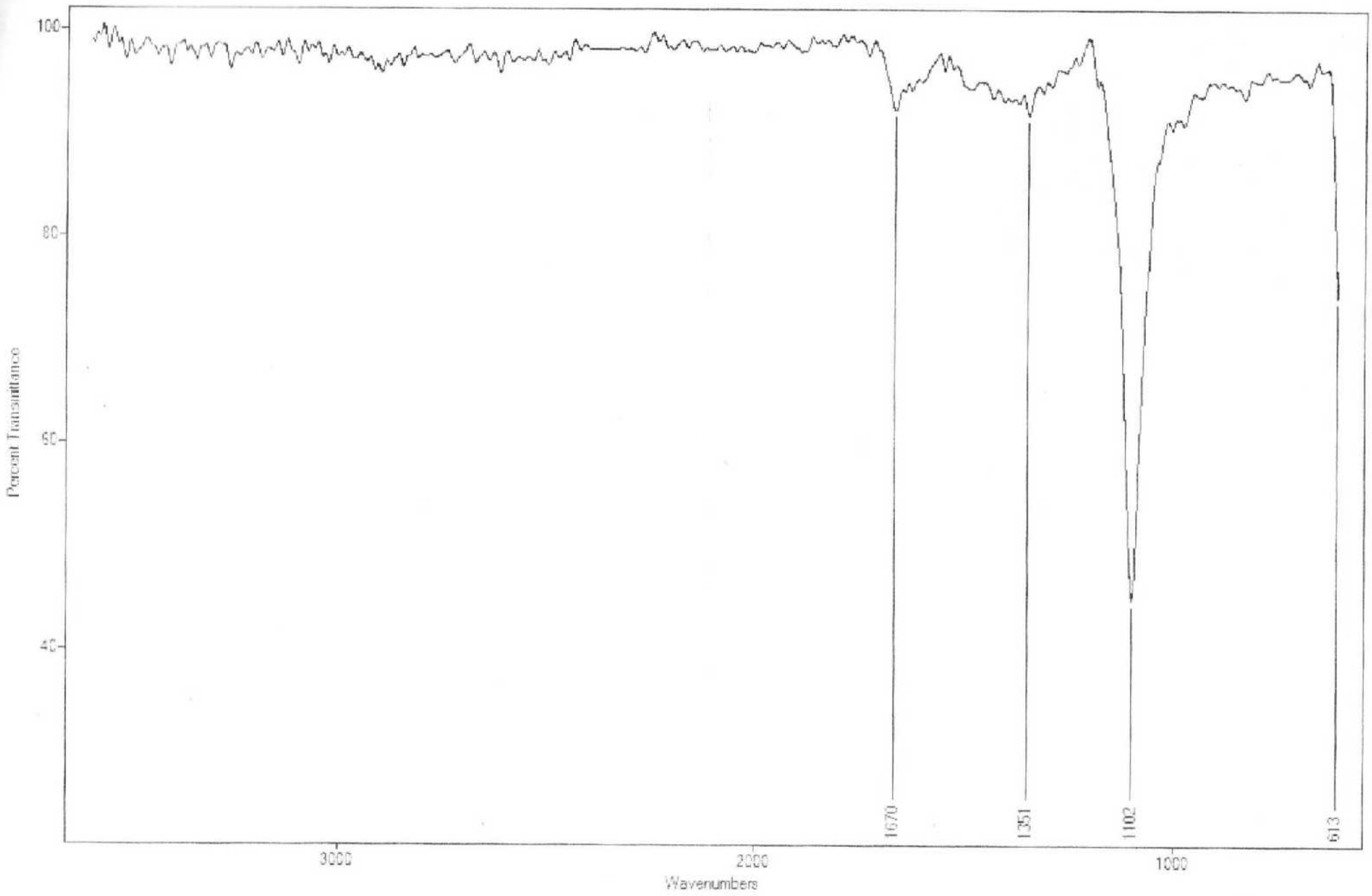
Tube No:	ORP	pH	Notes
1-5	~0.0mV	~7.4	KOH solution applied IR spectrum acquired.
1-8	~-250mV	~9.5	KOH Neutralized (ATR) The separation forms a precipitate

Notice that Tube 1-8 is largely the same, however, the peaks @ 836 cm^{-1} and 685 cm^{-1} become more pronounced.

These are the primary differences.

Protein LC: Tube 1-5 IR Spectrum

CDB Soluble Protein LC ATR Tube 1-5 OPR 0-0 pH 7-4 Dec 22 2018 - 01.spc: Synthetic spectrum by averaging. See audit trail for details.



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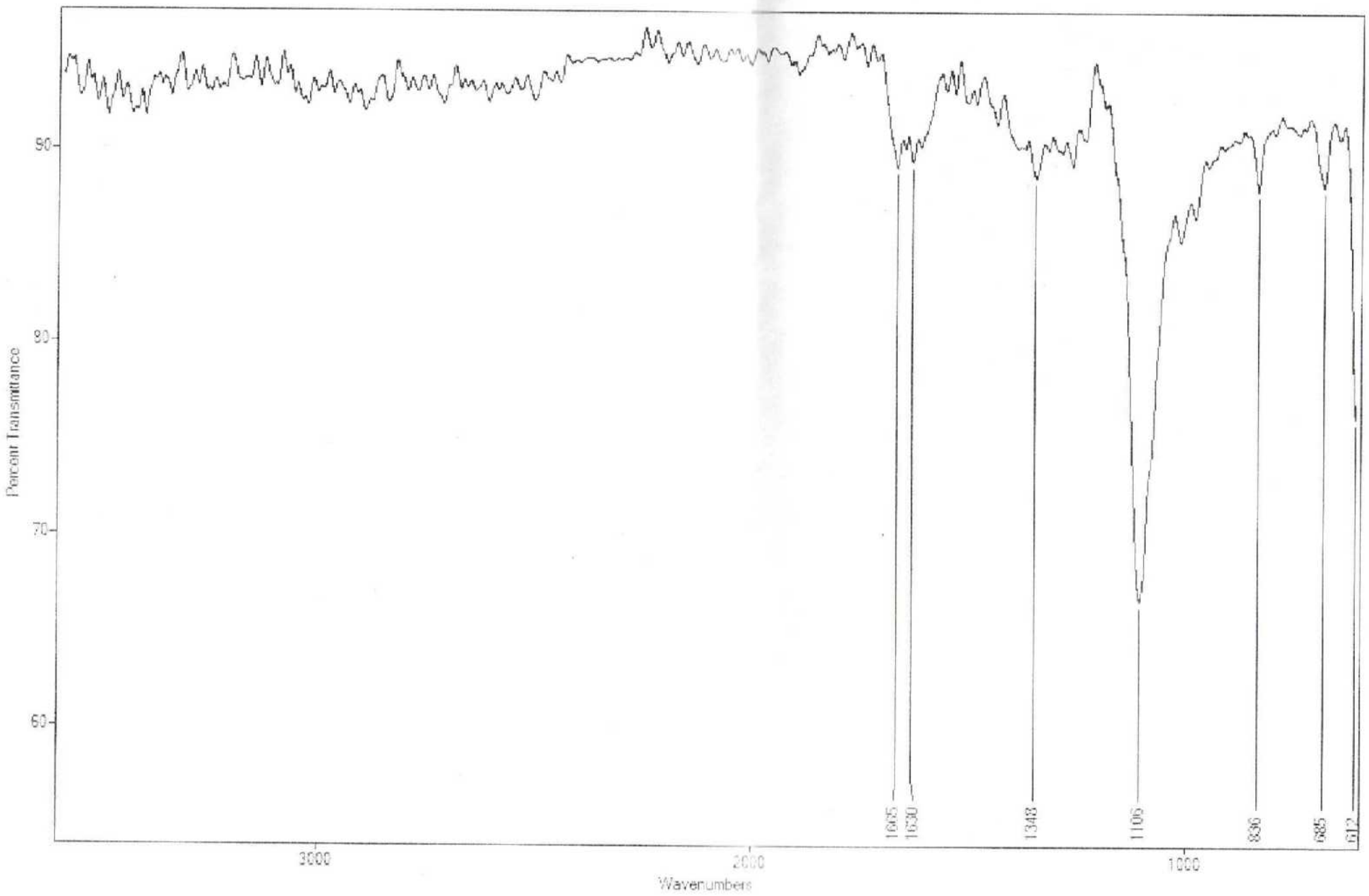
Protein LC: Tube 1-8 IR Spectrum



CDB Soluble Protein LC ATR Tube 1-8 OPR -250 pH 9-5 Neutralized Dec 22 2018 - 02.spc: Synthetic spectrum by averaging. See audit trail for details.



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Next we have

Tube 1	ORP	pH	Notes Vinegar In
2-2	-200mV	12.9	Neutralize pH for ATR purposes.

No precipitate formed upon neutralization, however. Neutralization weakens the intensity of the original orange color.

Actually, a precipitate does form gradually upon neutralization of the pH.

These solutions are very dilute and thus difficult to obtain a signal, hence the coarseness of the IR plots.

The method is to layer & evaporate the solution on the ATR crystal until a signal can be received.

Tube 2-2 introduces some significant changes, ie, Carbon is now in the picture.

A special interest in the likelihood of an aldehyde now appearing @ 2885 cm^{-1} as well as the potential Carbonyl group @ 1630.

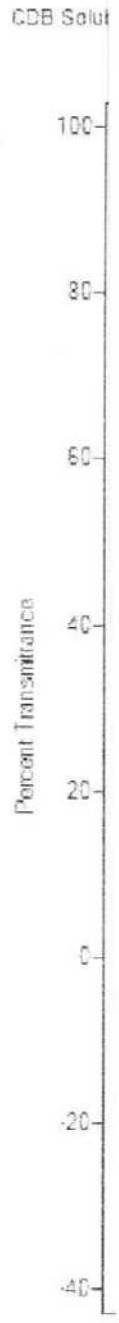
Aldehydes are indeed significant reducers hence the sharp drop in ORP during the separation period.

Notes last 2-10-19

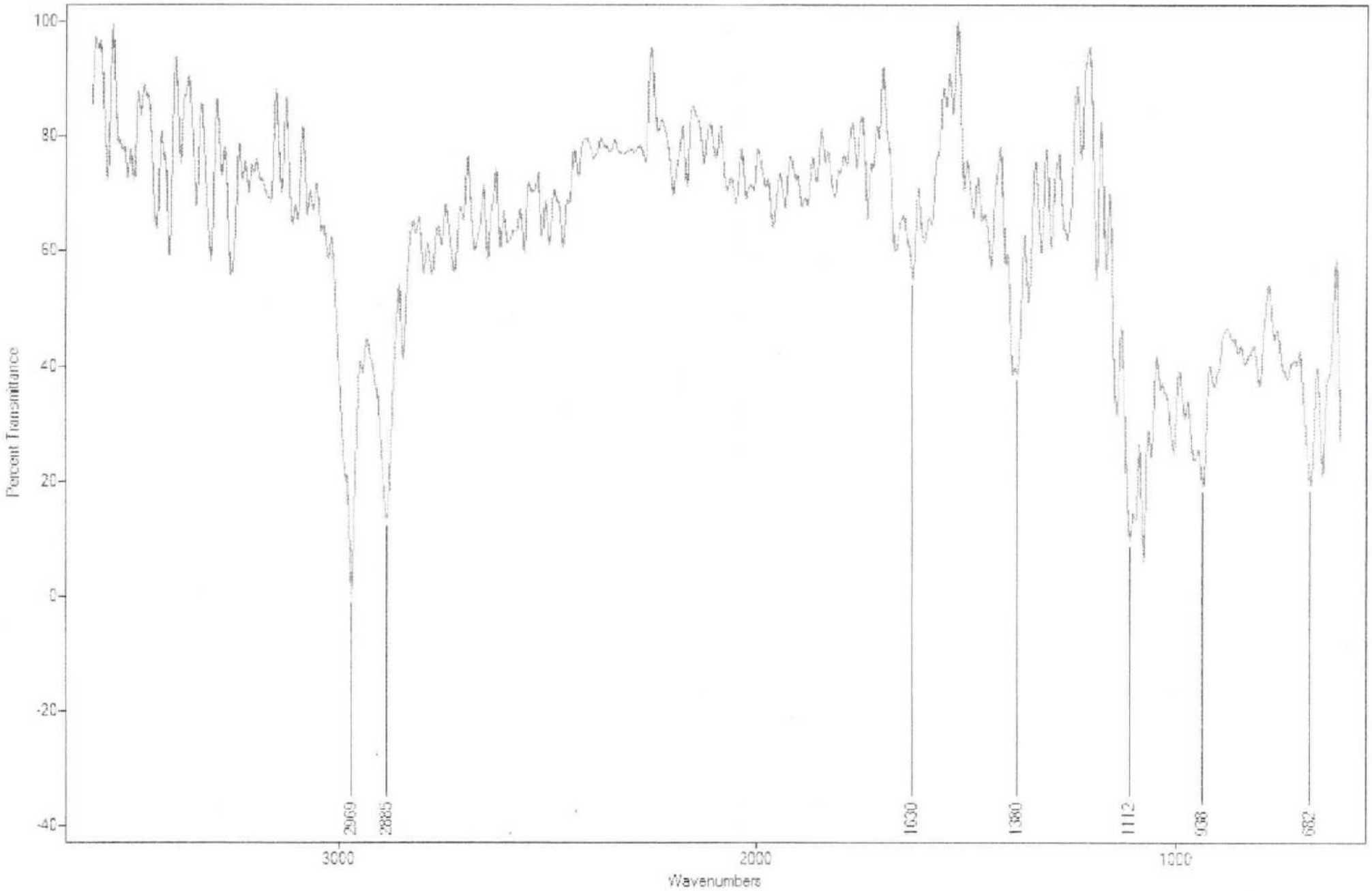
Aldehydes do change from ~~Not true~~ (Methyl Group) Notes 02-09-18

2705-2900 !! Problem LC: Tube 2-2 ~~IR Spectrum~~ Not true Possible C=C Bond, See Notes 02-09-18

Carbon a ~~Aldehyde~~, ~~Carbonyl~~ Appearance



CDB Soluble Protein LC ATR Tube 2-2 ORP -200 pH 12-9 Neutralized Dec 22 2018 - 03.spc: 12/22/2018 19:05:22 title



eFTIR

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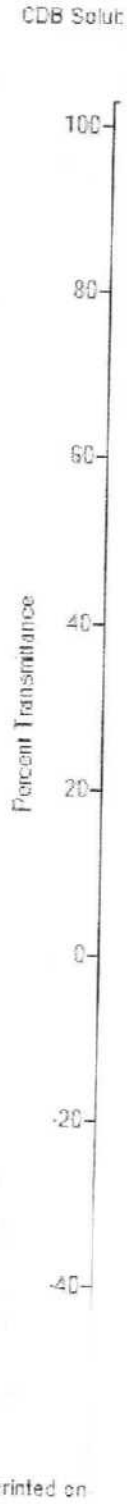
Caution here

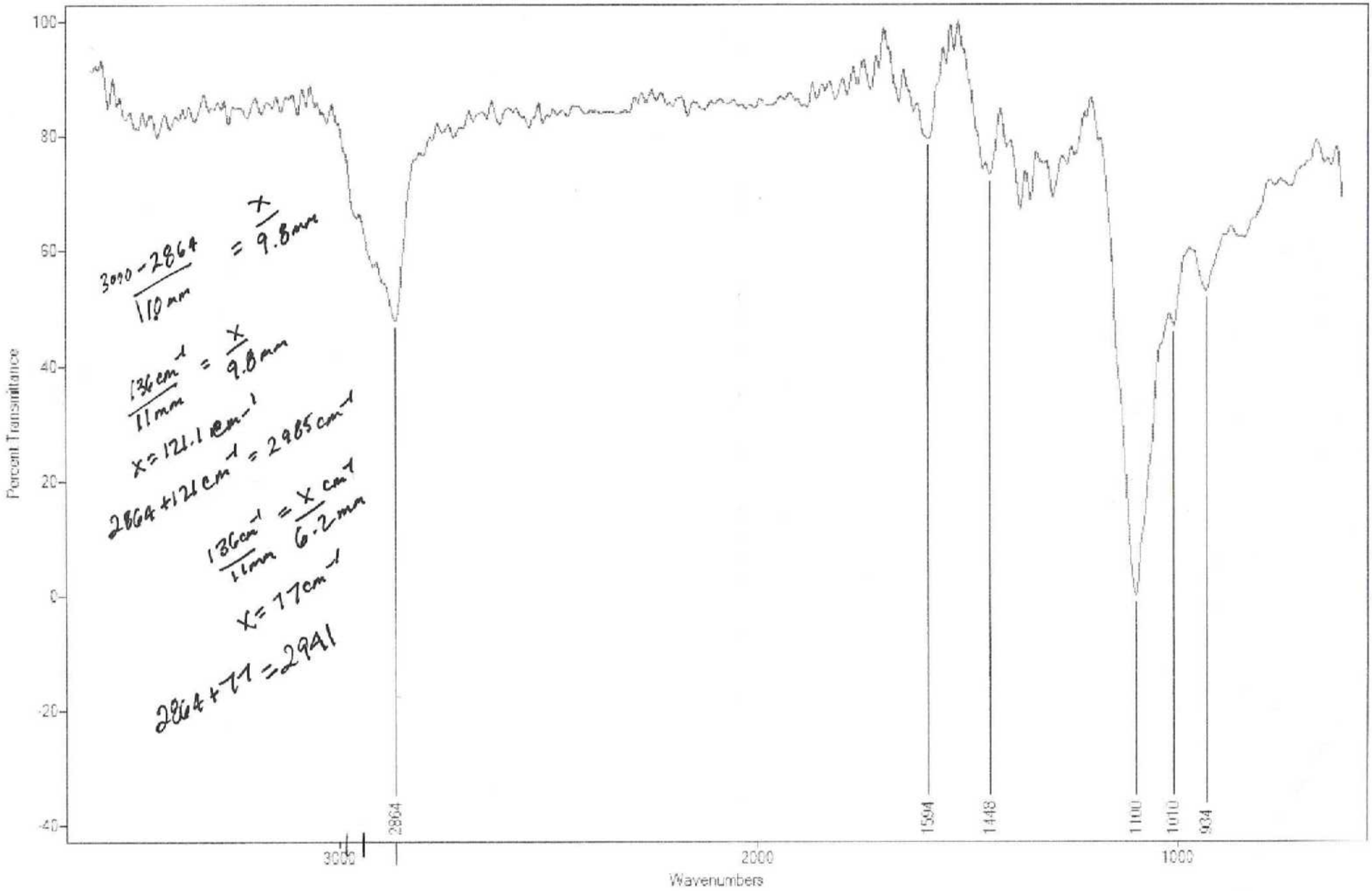
Not true. methyl Group.

Not so fast! 2-10-19 aldehydes do indeed range from 2705-2900!

Protein LC : Tube 2-3 IR Spectrum
Strong aldehyde Signal



CDB Soluble Protein LC ATR Tube 2-3 ORP -650 pH 12-9 Neutralized Dec 22 2018 - 04 spc: Synthetic spectrum by averaging. See audit trail for details.



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Tube 2-3 is already very interesting. The aldehyde signal looks to be especially pronounced

Conditions on Tube 2-3 are:

Tube	ORP	pH	Notes
2-3	-650 mV!	12.9	a/s. with Vinegar

Neutralized for ATR. No strong precipitate visible in neutralized solution. Some precipitate does form & settle in original collection tube. Rapid evaporation on ATR

The presence of apparently a strong aldehyde component and the especially low ORP value are distinctive in the separation

The separation was the last eluted with vinegar. & gradually the increasing ORP to ~ -130mV is when the separation settles out.

We have now switched to HCl in a moderate strength. We have seen the tinge of purple eluted under these conditions. Our result are:

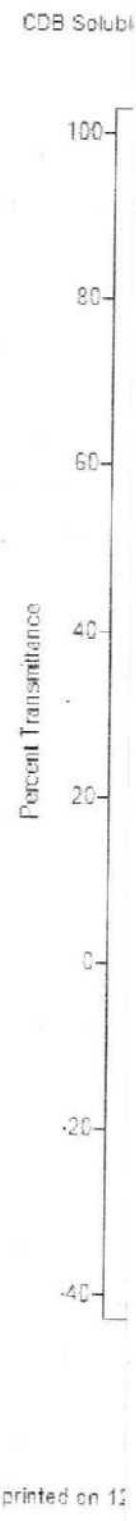
Tube	ORP	pH	Notes
2-5	-7mV	12.1	Tinge of purple has shown

Slow evaporation on ATR

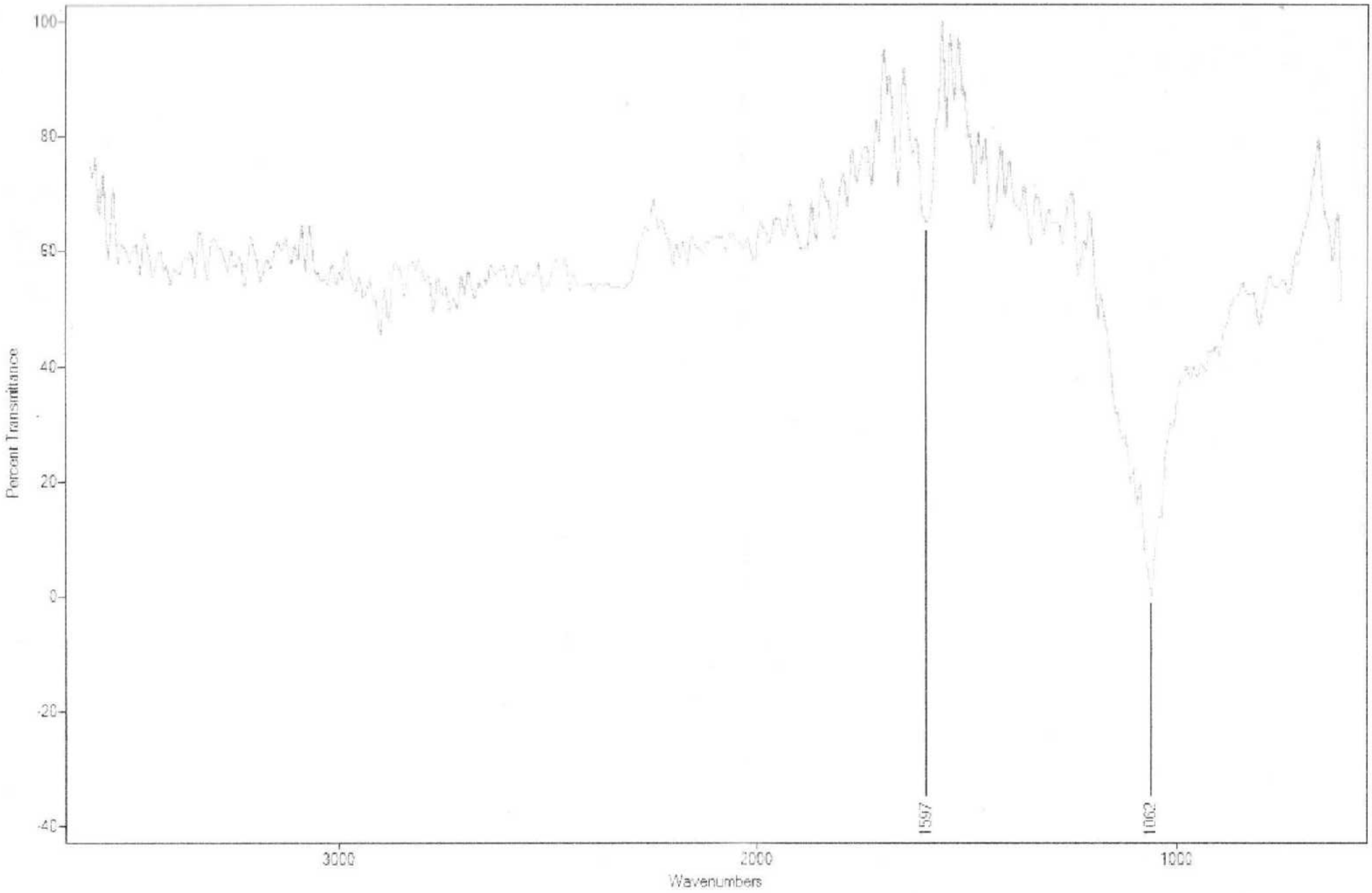
Notice pH is still highly alkaline even though ORP has steered to a light value

Very sensitive to pH changes. No buffering capacity

Protein LC: Tube 2-5 IR Spectrum
End of Run



CDB Soluble Protein LC ATR Tube 2-7 ORP -07 pH 12-1 Neutralized Dec 22 2018 - 05.spc: 12/23/2018 01:13:52 title



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With tube 2-5, our run is complete.
The column appears to be packed.
The column is reasonably clean looking
and all colored compounds appear to have
eluted.

The column has actually become blocked essentially
w/ the use of moderately strong HCl. There
may be a weak precipitate that is remaining
in the column. The HCl has been removed
and the eluate replaced with vinegar.

We do have extremely slow flow taking place
again but the column does require flushing.
I will work w/ vinegar and KOH @ the
time to work on flushing the column.

—

Vinegar seems as good as anything right now.
KOH did not improve flow.

NOT SO GOOD Clr

What about bleach: Vinegar and bleach have
been combined into the column.

—

Ok, I have the column flowing very well again.

The answer was two fold: NOPE

NOT SO GOOD Clr.

1. Vinegar + bleach do indeed make a powerful
   cleaning combination.
2. Inserting a wire upward through the nozzle
   to open up the encrustment in the
   sand filter.

Fact is, the column now looks better than it has for some time.

The column and the sand (top & bottom) are all being bleached & cleaned to a solid white color. All residual colors and bands are being removed cleanly

NOPE! Ch2! ~~There is a great deal of oxidation taking place w/ the Vinegar-bleach combination.~~ NOPE

Along the way I did try H_2SO_4 (4.5) even with additional (cautious) heating of the column, but this did still not produce a breakthrough or flow. The wire insertion did make the difference; an encrusted plug existed @ the bottom of the column.

We are in excellent shape again. Now we head towards flushing the column with H_2O runs after this all passes.

Dec 23 2018

I have run an interesting test w/ the sample tube containing an aldehyde. I have heated it up. I attempted to determine the boiling point but this did not fare too well because of overheating and inadequate pressure release.

I did learn, however that the boiling pt departure from H_2O is not highly significant. This was unexpected, as this sample evaporates very rapidly on the ATR plate so I expected a low boiling point.

Nevertheless, subjecting Tube 2-3 w/ the aldehyde to heating

DID not remove or appear to alter the aldehyde group. It is still there as shown on the IR spectrum on the next page.

We most definitely have an aldehyde. Aldehydes are commonly toxic and interfere w/ glutathione uptake & the detox process

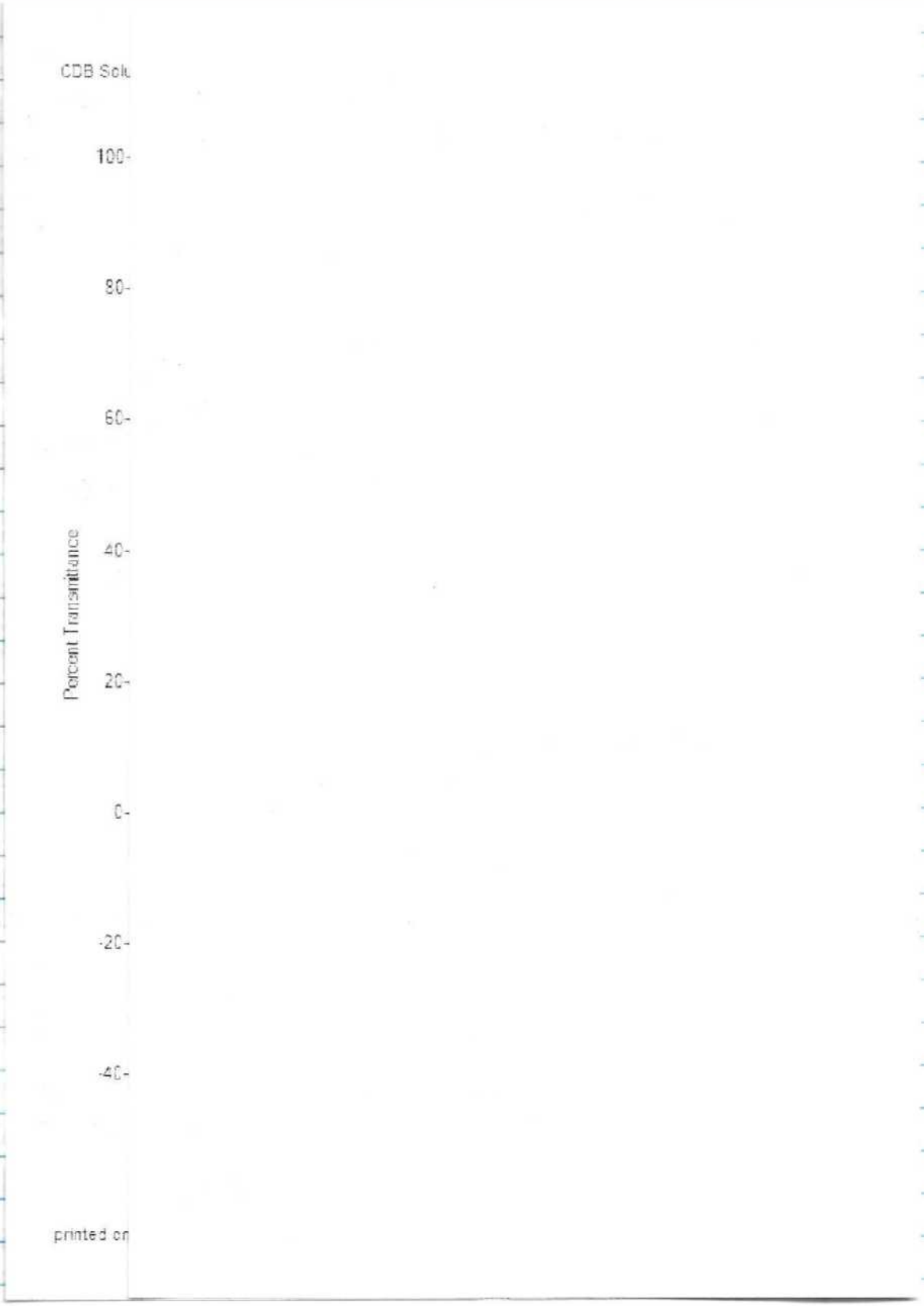
We do see that variations in the compound do take place after heating, but these are occurring in the fingerprint region.

Note — Aldehyde conclusions of existence ARE NOT warranted @ this stage. — Note

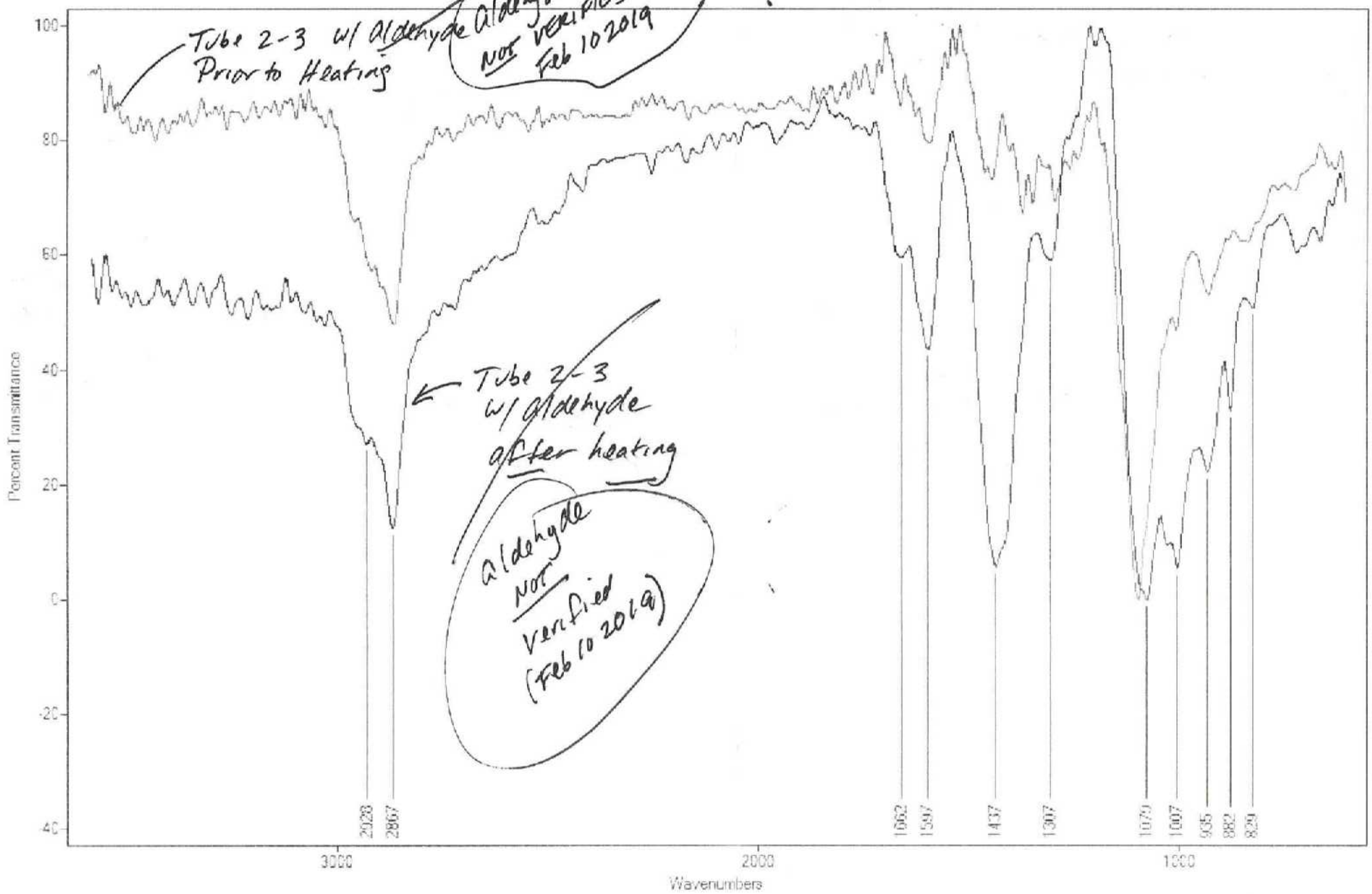
* See note Feb 16 2019

Aldehyde Tube Post Heating

Protein LC Tube 2-3 IR Spectrum:



CDB Soluble Protein LC ATR Tube 2-3 ORP -650 pH 12-9 Neutralized Heated Dec 22 2018 - 06.spc: Synthetic spectrum by averaging. See audit trail for details



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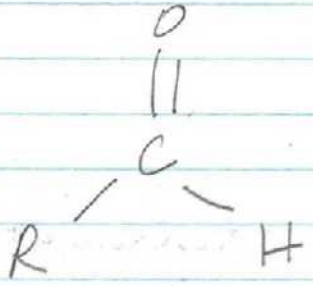
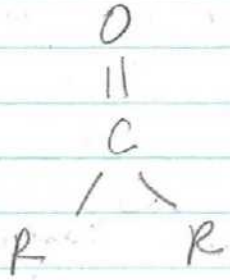
An aldehyde structure is

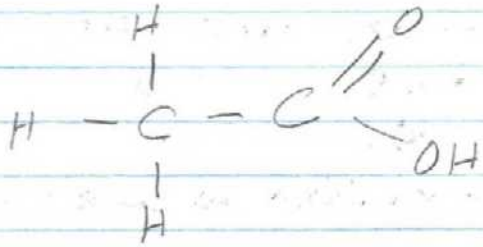


Aldehyde



Ketone

* KOH and then vinegar were used in the LC process.



Acetic Acid Structure

Acetic acid is the second simplest carboxylic acid. Acetic acid apparently does not have an aldehyde group within it so we definately have a separate process here.

* The properties and toxicity effects from aldehydes is a very important topic.

Certainly deserving of a separate paper.

Dec 24 2018

1. Make another LC run on the protein
2. Capture molality reading
3. Backup IR spectral data

ORP & pH (separate electrodes) serve as very good detection of change in the LC column elution. Actually more revealing than a UV detection alone would be.

Tube	ORP mV	pH	Notes
3-1	+250	7.6	KOH added, light green
3-2	-140	7.1	Light Rust Color, KOH
There is a purple-bluish cast that appears in the column here.			
3-3	-65	7.3	Very Light Rust Color, KOH
3-4	-185	7.6	Vinegar, light pink
With vinegar, the bluish cast is definitely being pushed down the column quite rapidly.			
3-5	-125	5.7	Vinegar ΔORP occurring Light yellow
This is the tail of the purple cast captured. ORP is starting to increase rapidly now			
3-6	+215	4.4	Vinegar, clear
Column looks clean.			smells like vinegar also
3-7	+300	+4.3	Vinegar, clean

Everything says there were residual materials in the column during the previous run. You require repeatability. Aldehyde observations are also in question & subject to review.

* Note this 02-14-19

Thus these pH values make a whole lot more sense.

Ok, there is quite interesting. Tube 3-1 is important: It is the protein study, possibly in even a more purified form.

Here is what we see:

1. The original protein is brown in color. This says the iron in the protein is in an Fe^{+2} state.

2. I believe the previous column run may have had some residual material in the column that distorted the results. Notice that the ORP and pH values never reached the earlier set of more extreme values, ie -650 mV on the ORP and pH of 7.12. This should settle nicely out if we can make an additional run.

3. Tube 3-1 evaporate to a thin shellac like film layer on the ATR plate, exactly like we have always seen with the protein. Keep in mind we also know that we have a reducing sugar in the protein solution, this could account for some of the viscosity issues as well as the film format on the ATR plate.

4. ~~The~~ Notice that the protein eluted immediately from the column. Also notice that it was light green, which indicate a reduction to Fe^{+2}. Now also recall that the eluent is KOH. We also know the original protein is highly acidic, i.e., ~ 3.6

Therefore we have strong indications of both acid-base chemistry & redox chemistry.

I have run a test directly adding weak KOH to the brown (oxidized) protein. It immediately turned the protein green, once again indicating that we have both acid-base & redox chemistry involved here.

This is a rich topic for research in the future.

5. Notice in the IR spectrum that we

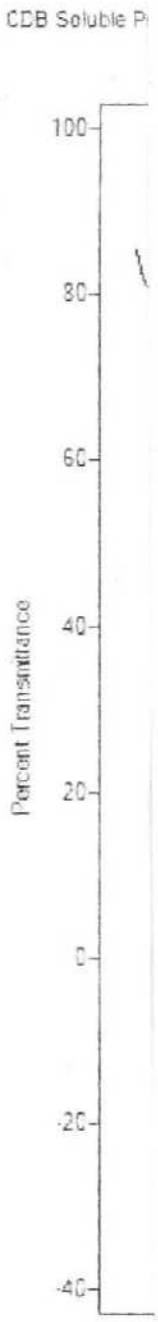
Continue to show the presence of an aldehyde

w/ absorbance @ 2886 cm^{-1}. This is important; I am not sure if previous plots demonstrated this.

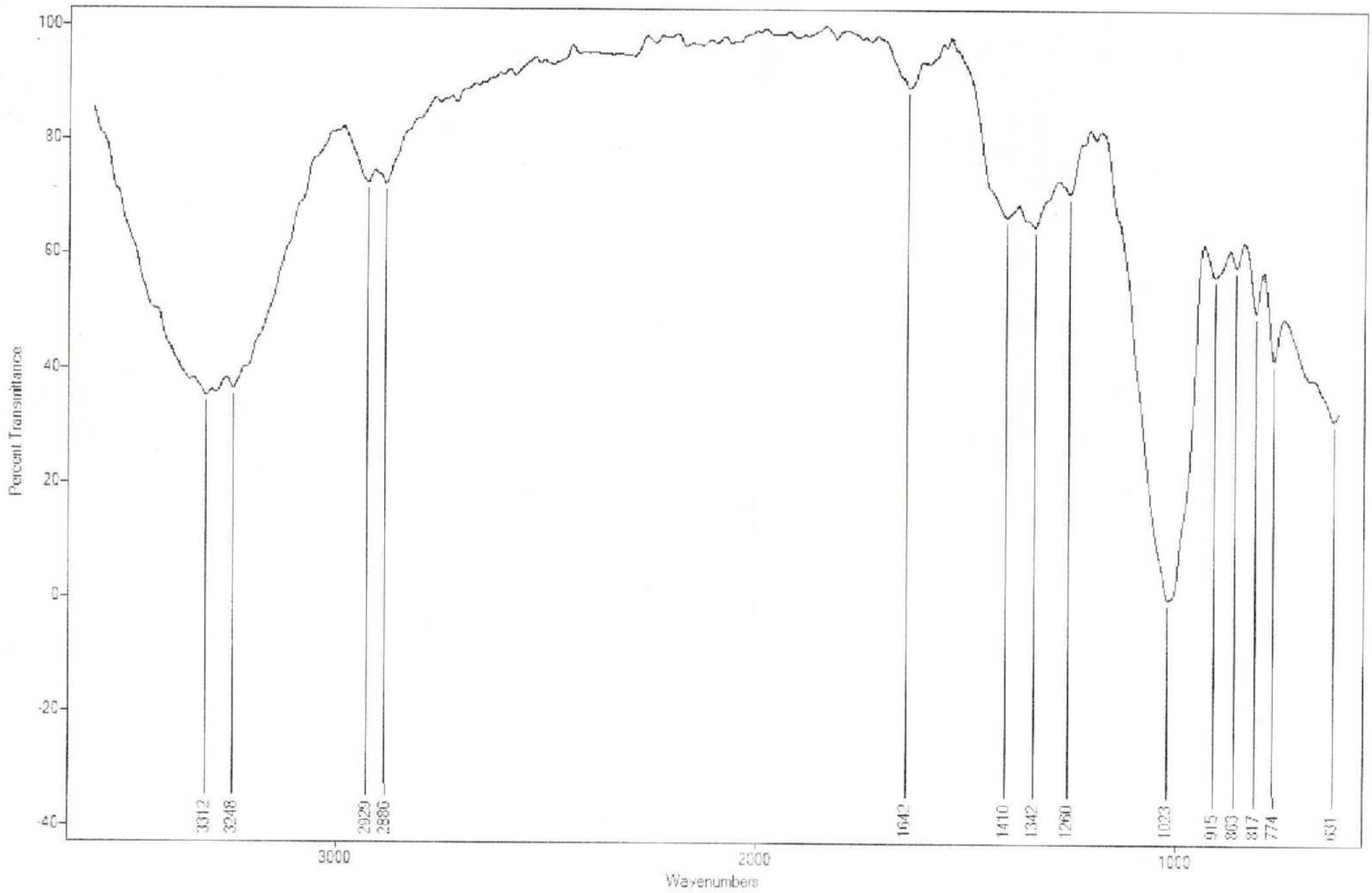
6. Notice also in the IR the likelihood of double peaks in the amide region, the very weak carbonyl area, & the exceptionally strong absorbance @ 1023 cm^{-1}.

Aldehyde Contents to be present

Protein LC Tube ~~2-3~~ 3-1 IR Spectrums



CDB Soluble Protein LC ATR Tube 3-1 OPR 250 pH 7-6 Dec 23 2018 - 07.spc: Synthetic spectrum by averaging. See audit trail for details.



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The process by which the final eluate has come out first rapidly from the column, the fact that the eluent is KOH, the fact that it is substantially the protein, and any differences in the IR spectrum from the "raw" or original CDB soluble protein are all very interesting topics.

—

Because of the vinegar aroma, the pH @ acid, & the eluate being clear, we assume the column is also clear @ the time.

—

Now time to flush w/ H_2O.

—

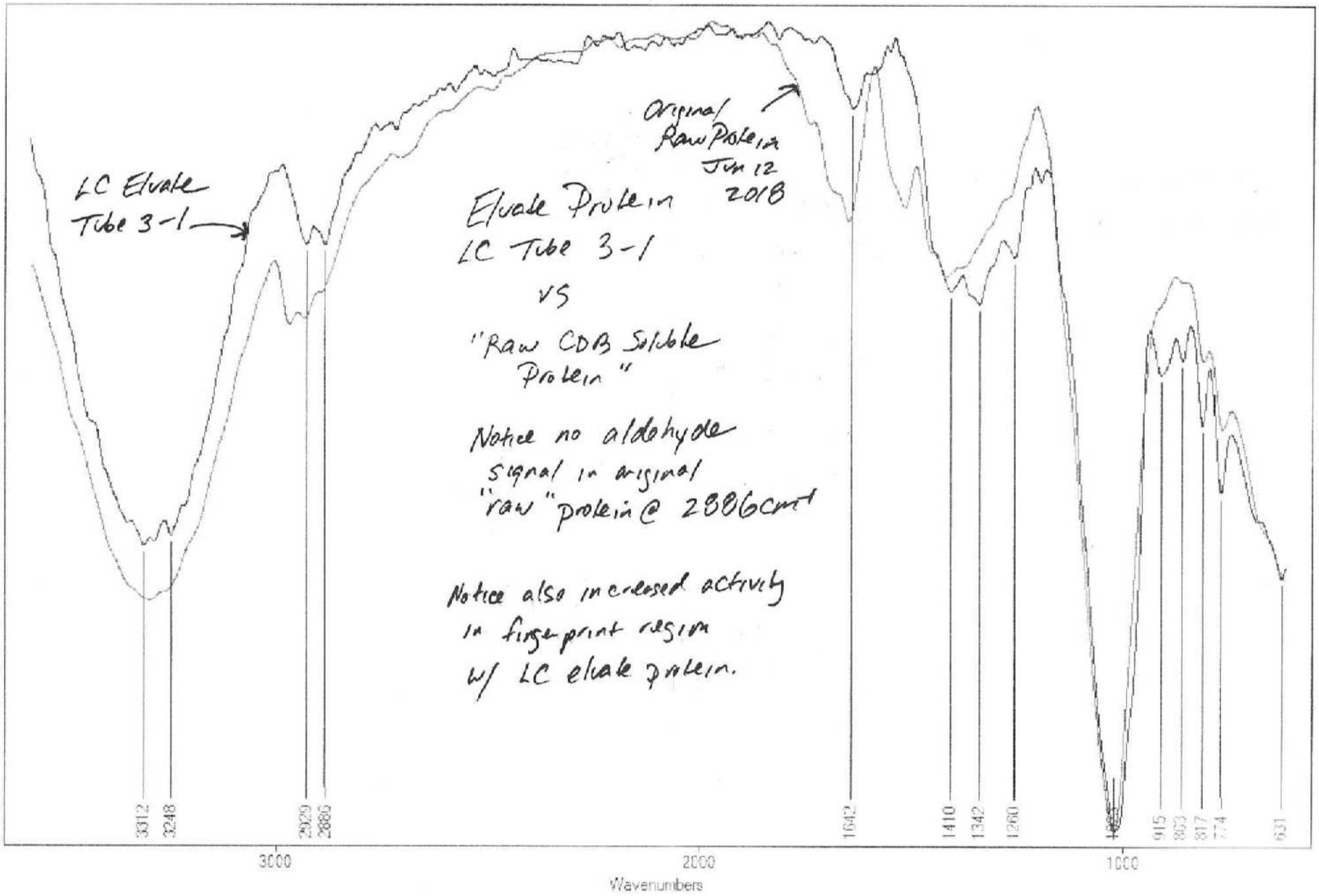
Note: the addition of KOH to the raw soluble protein forms a very dark green precipitate.

Comparison of LC: Tube 3-2 against native protein form
Aldehyde detection in LC elvate.

CDB St

printed c

CDB Soluble Protein LC ATR Tube 3-1 OPR 250 pH 7-8 Dec 23 2018 - 07.spc: Synthetic spectrum by averaging. See audit trail for details



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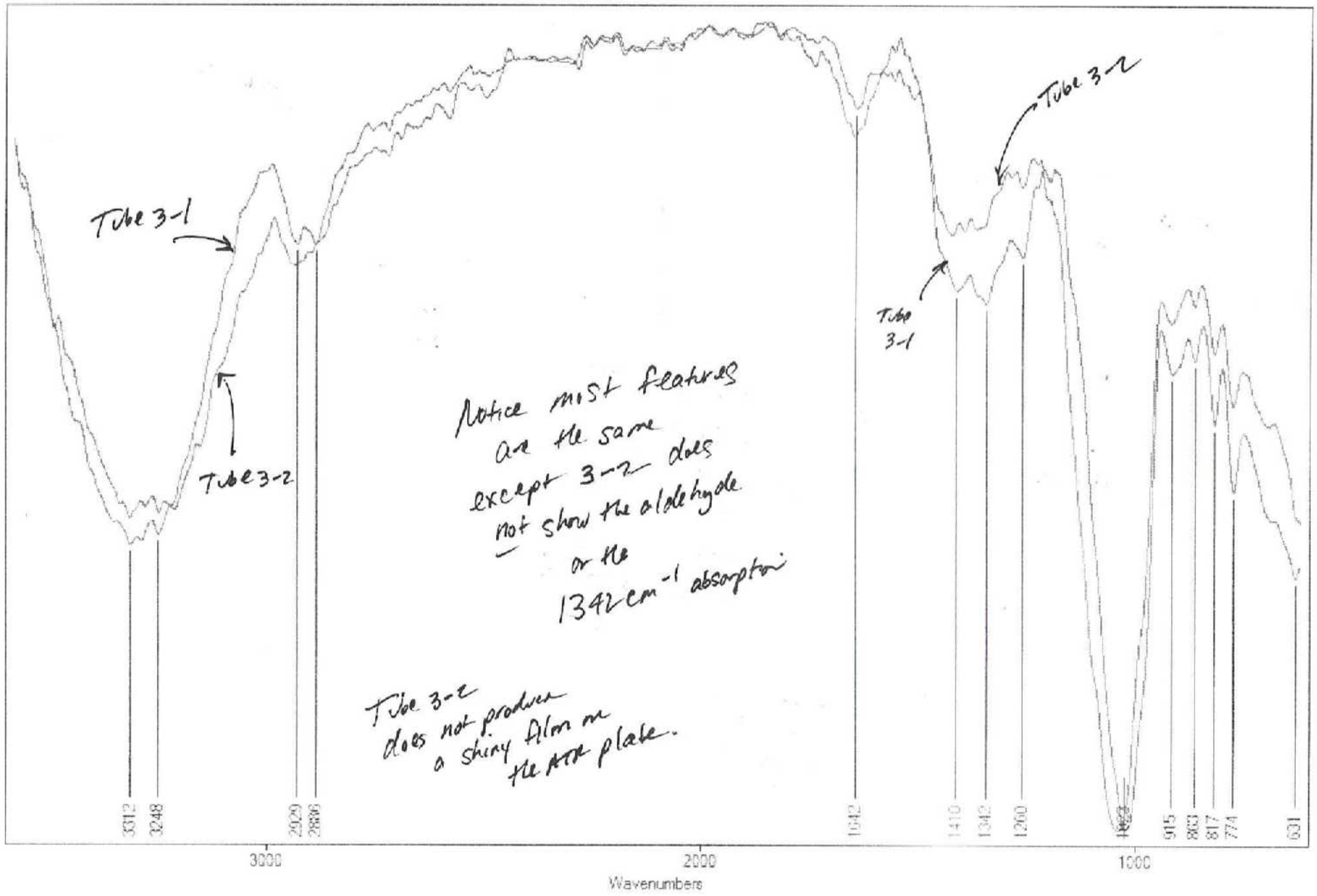
LC Tubes 3-1 & 3-2 Compared IR spectrum

CDB

printe

CDB Soluble Protein LC ATR Tube 3-1 OPR 250 pH 7-8 Dec 23 2018 - 07.spc: Synthetic spectrum by averaging. See audit trail for details



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In Tube 3-4 we now see an entirely different compound is emerging.

This should be capturing the bluish cast in the column that was referred to.

Notice the Eluent is now Vinegar in the case.

A Carbon signal has been brought into the picture and the aldehyde is no more.

Massive absorbance @ 1568 cm^{-1} · Candidates amide, carboxylate
also 1393 cm^{-1} sulfonic ester
and very strong @ 1114 cm^{-1} sulfone, pthalic ester
thio amide

—

Tubes 3-6 & 3-7 do indeed seem to be generally clear of compounds and are at the tail of the run.

Tubes 3-1 & 3-4
appear to be the most important & identifying

In tube 3-1 we have confirmed the presence of an aldehyde.

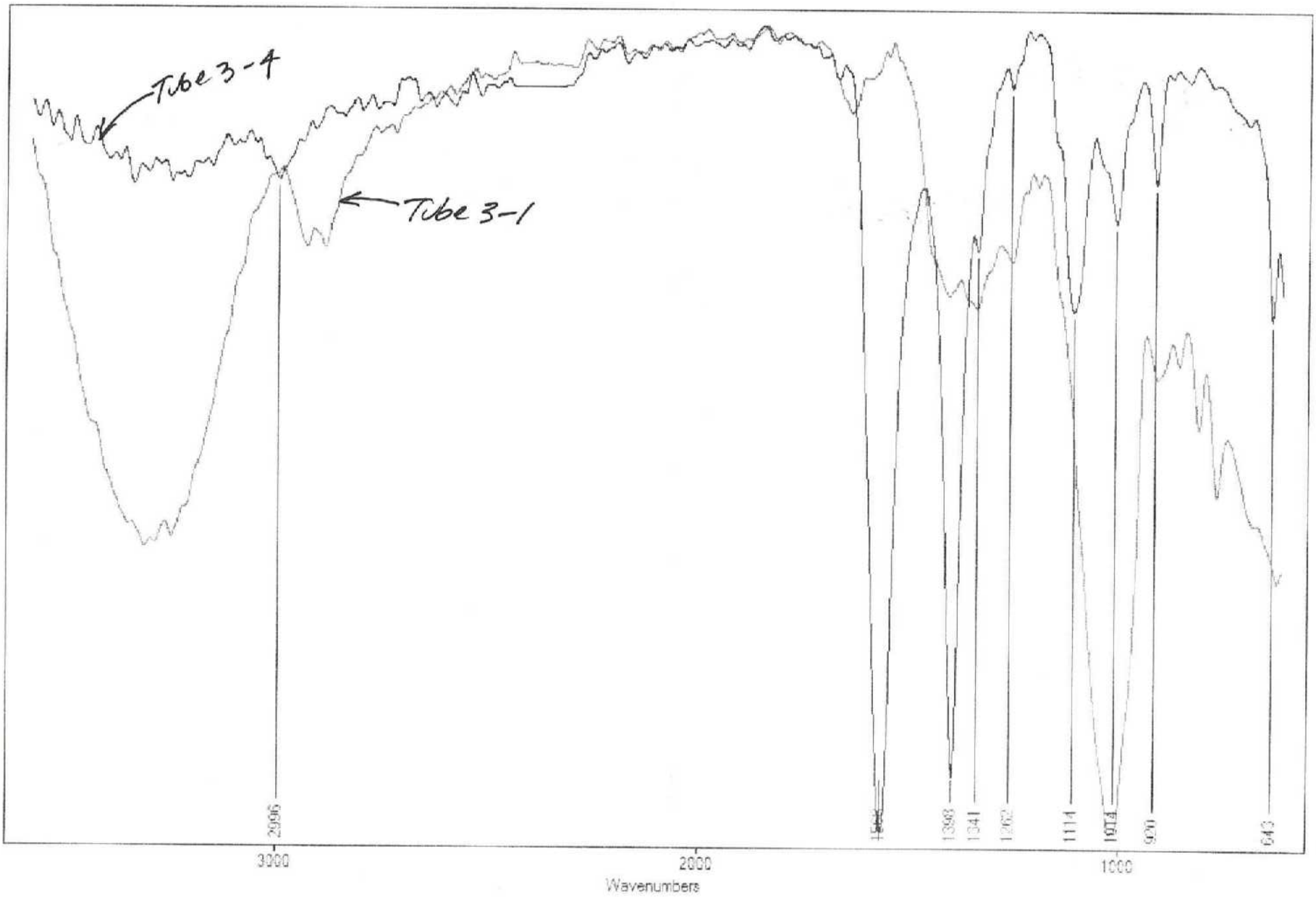
It would indeed be beneficial to repeat the one more time to seek stability in the results.

Protein LC: Tble 3-4 Entirely different compound emerging.

CDB Sc

printed c

CDB Soluble Protein LC ATR Tube 3-4 OPR -185 pH 7-8 Dec 23 2018 - 09.spc: 12/24/2018 14:37:29 title



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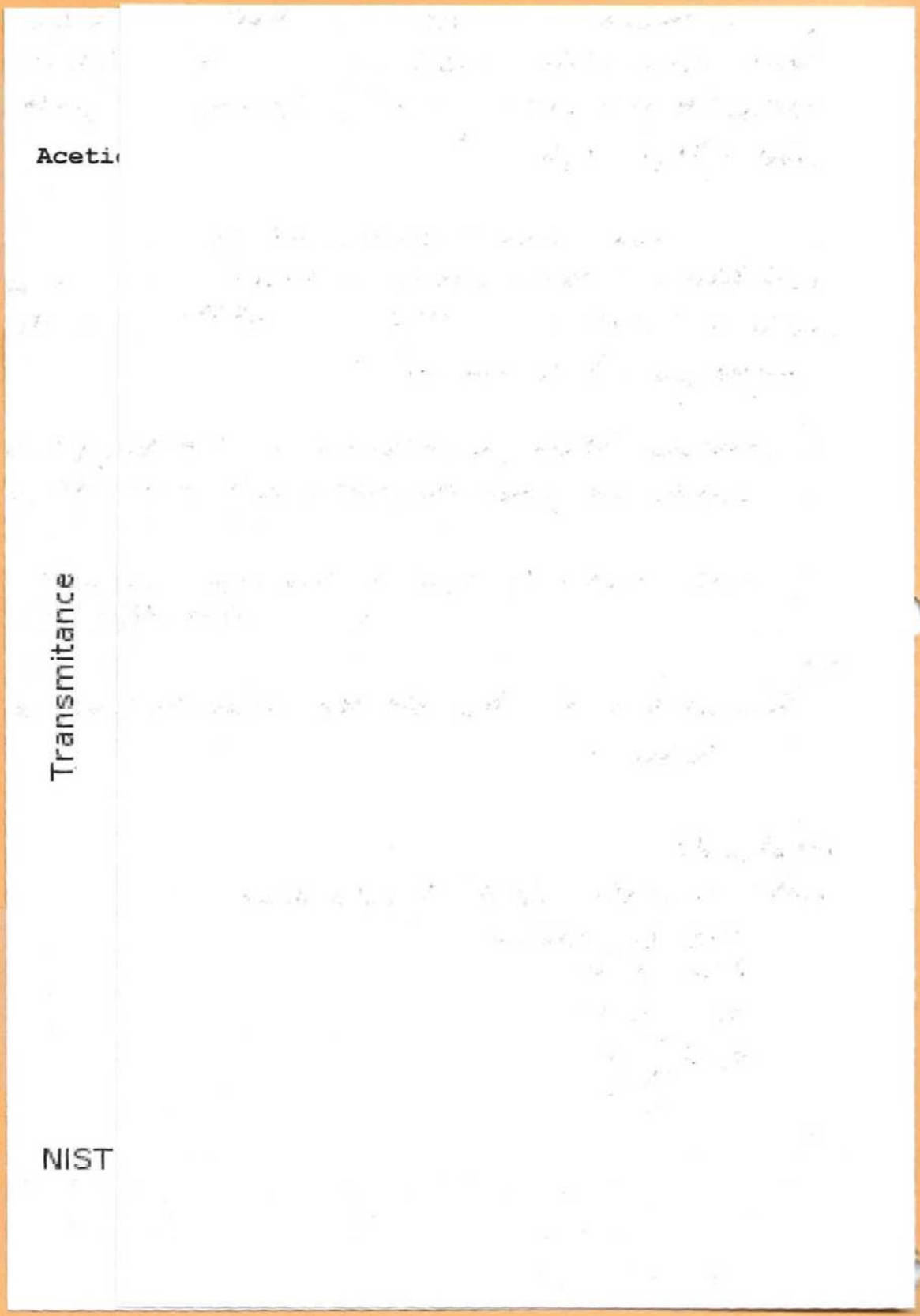
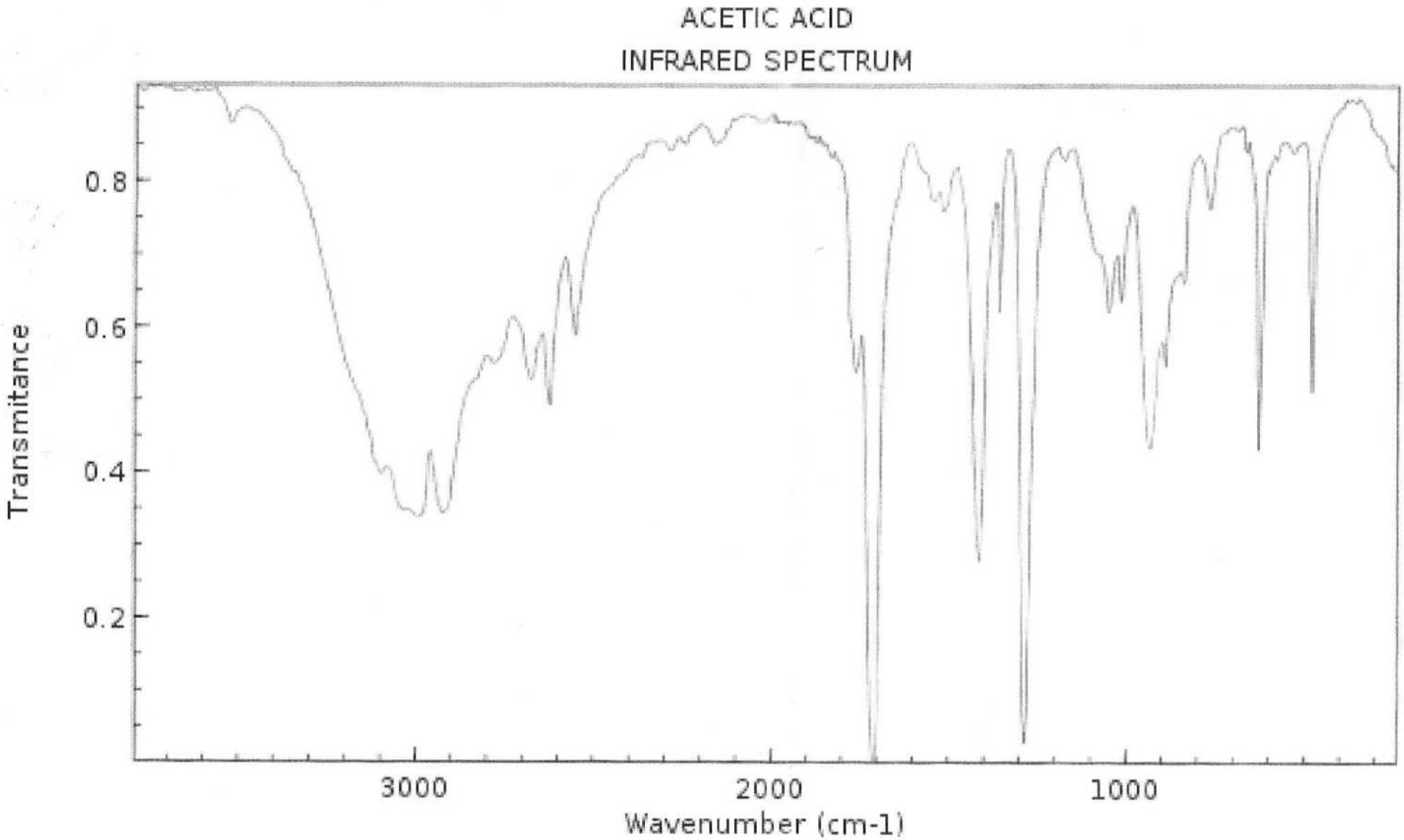
Acetic Acid Reference Spectrum



Acetic Acid (liquid) reference spectrum



NIST Chemistry WebBook (https://webbook.nist.gov/chemistry)

I have learned some very important lessons w/ LC today. Your column was blocked again. The cause is too strong of an base will precipitate the protein and clog the column.

If you are going to work w/ acid-base chemistry (or any eluent for that matter) test it prior to make sure that you are not going to precipitate and plug up the column.

I have reworked the column. It was probably due regardless but try not to plug it up again.

We need to start w/ H_2O & then only a mild base, just enough to neutralize the protein (even less) which is @ pH 3.6. Use mild eluents to begin with and then increase strength cautiously as required.

Next set:
Tube 4-1 shows the aldehyde again
4-2 Can pass
4-3 Pass
4-4 Pass
4-5 Pass
4-7 Pass

"Pass" means no new significant information available from the IR spectrum or detector information.

Tube	ORP	pH	Notes
4-1	+150	7.2	H_2O, light yellow.
4-2	−35	6.6	H_2O, light yellow-green
4-3	+110	6.8	H_2O, light green
4-4	+30	~~+3.0~~ 6.8	H_2O, clear

Nothing is expected from here.
Switch to acetic acid add HCl

4-5	+435	+3.0	Vinegar - slowed column

The slowing of the column here is a surprise. The protein is soluble in vinegar, & do not know why the column would slow down other than it is now longer than before.
Let's add some HCl
Then has improved flow rate some, but it is still slow.

I have neutralized the pH. It has turned green w/ some precipitate as well.

4-6	−126	6.7	Mild KOH added to

bring pH towards neutral. Flow rate may have increased slightly. Notice negative ORP Light Green Yes, flow rate is much better towards neutral pH. Possibly make only very slight pH changes. Never let pH go above neutral though?
Earlier,
But aldehyde came in strong @ pH ~ 12!

Tube	ORP	pH	Notes
4-7	-85mV	7.1	Mild KOH added toward neutralization. This has been sitting a while now and has oxidized and turned to a rust color.

—

OK, I think we have a reasonably representative set of IR spectra to represent the LC work that has taken place.

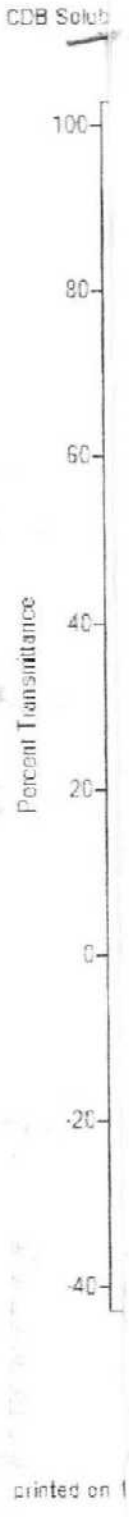
The most significant upshot understood so far is the repeated documented presence of an aldehyde. This raises potential for significant toxicity issues. *

In terms of LC work, there is a lesson of heavy gradual + caution w/ respect to alteration in the eluent - extreme changes can block the column fairly quickly if you are not careful.

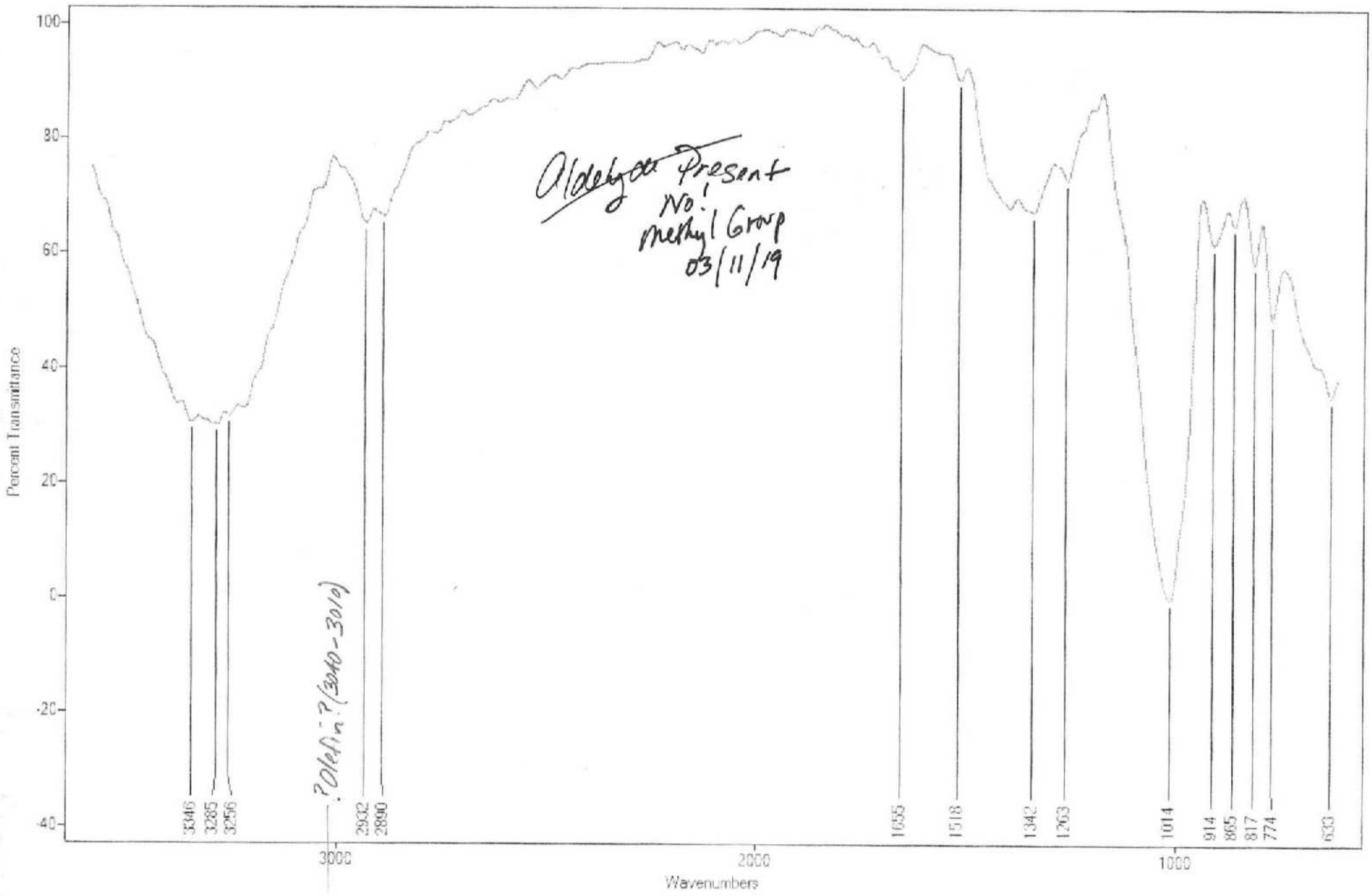
Also check precipitation effects of the eluent prior to introducing into the column, not after!

Example, ammonia is indeed causing some precipitation, any base is having this effect.

LC Protein: Tubes 4-1 & 4-6 IR Spectrum



CDB Soluble Protein LC ATR Tube 4-1 OPR -150 pH 7-2 Dec 23 2018 - 10 spc: 12/24/2018 17:28:23 title Tube 4-1



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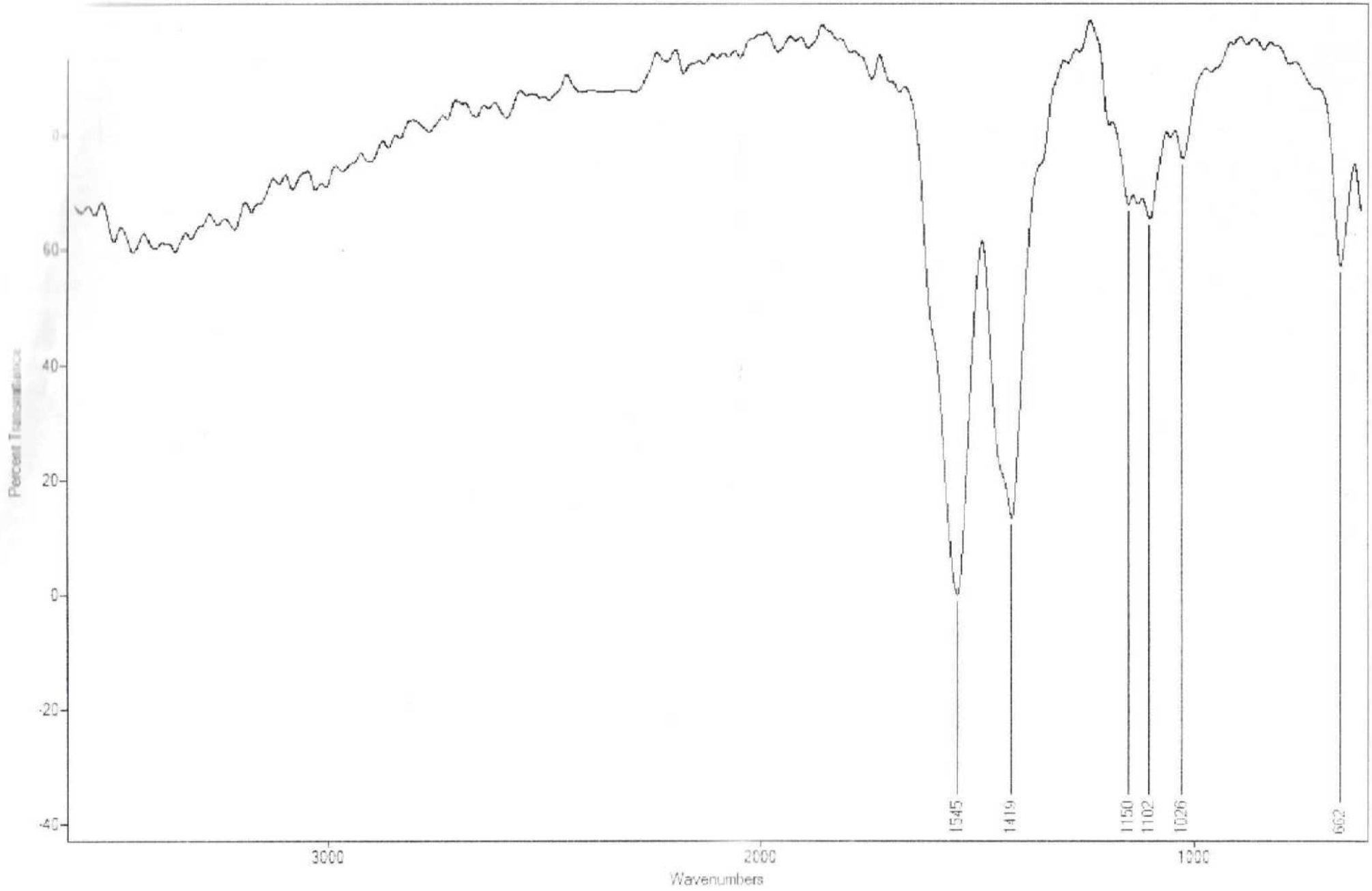
Protein LC ATR Tube 4-6 OPR -125 pH 6-7 Dec 23 2018 - 11.spc: Synthetic spectrum by averaging. See audit trail for details.



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Ammonia produces a green precipitate
Bleach produces a bold yellow color, no precipitate
Any base produces a green precipitate.

The bleach reaction is a very interesting one (color)
Bleach increases the ORP dramatically.
Small, rapid drops on the column w/ 50% bleach. ORP +700.
Question: what would act as a reducer?

Sodium & Magnesium are strong reducing agents.

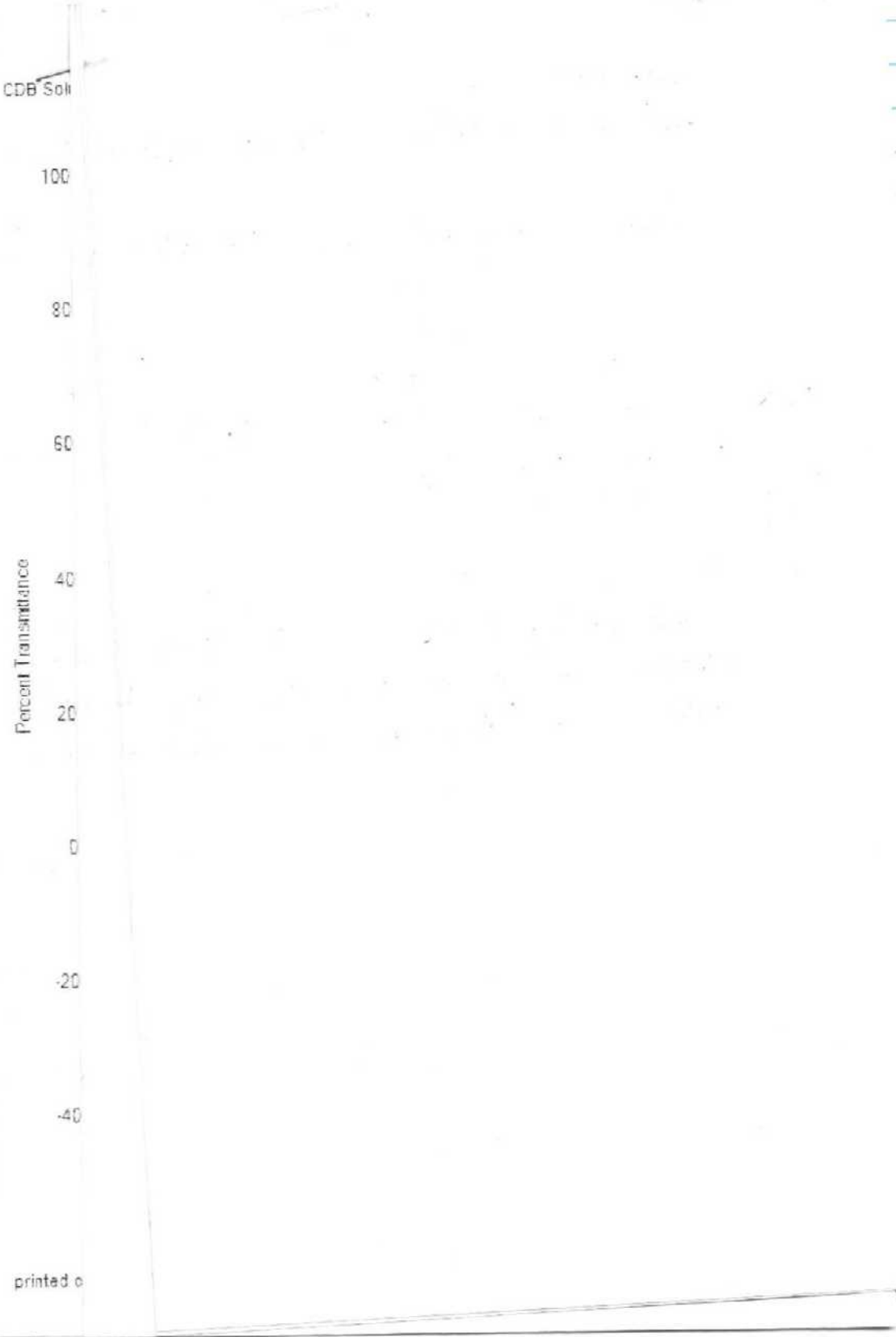
Ok, something very different has been done now, and we have a very different result because of it.

I have decided to try $MgSO_4$ as the eluent.
Fascinating results.
The column has turned blue-grey in color.
We have produced a reduced ORP state, which was the primary goal.
Our data is:

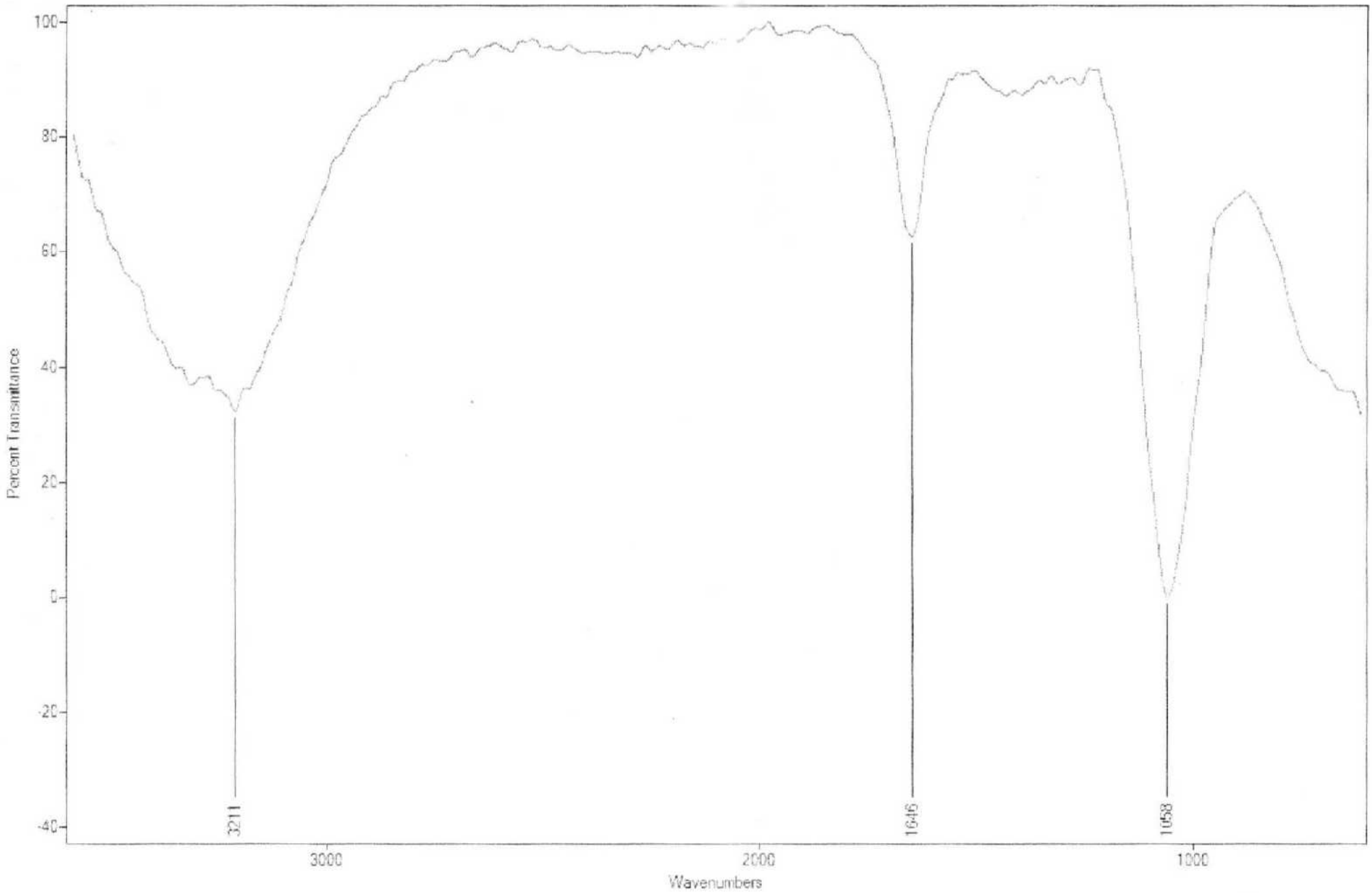
Tube	ORP	pH	Notes
1-1	-200	7.7	$MgSO_4$ eluent

We also have a very clean and simple IR spectrum, and it is different. Preliminary analysis using Parker tables strongly suggests that I have an "imine salt" with a phosphorus ester. Spectrum follows, also w/ a SDBS search, which also indicates we most certainly have an amine.

LC Problem: Tube 1-2 (DEC 24)
Without source doubt we have an amine.
Possible: Amine salt w/ phosphorus. esters.



CDB Soluble Protein LC ATR Tube 1-2 OPR -200 pH 7-7 Dec 24 2018 - 12.spc: 12/25/2018 02:14:59 title

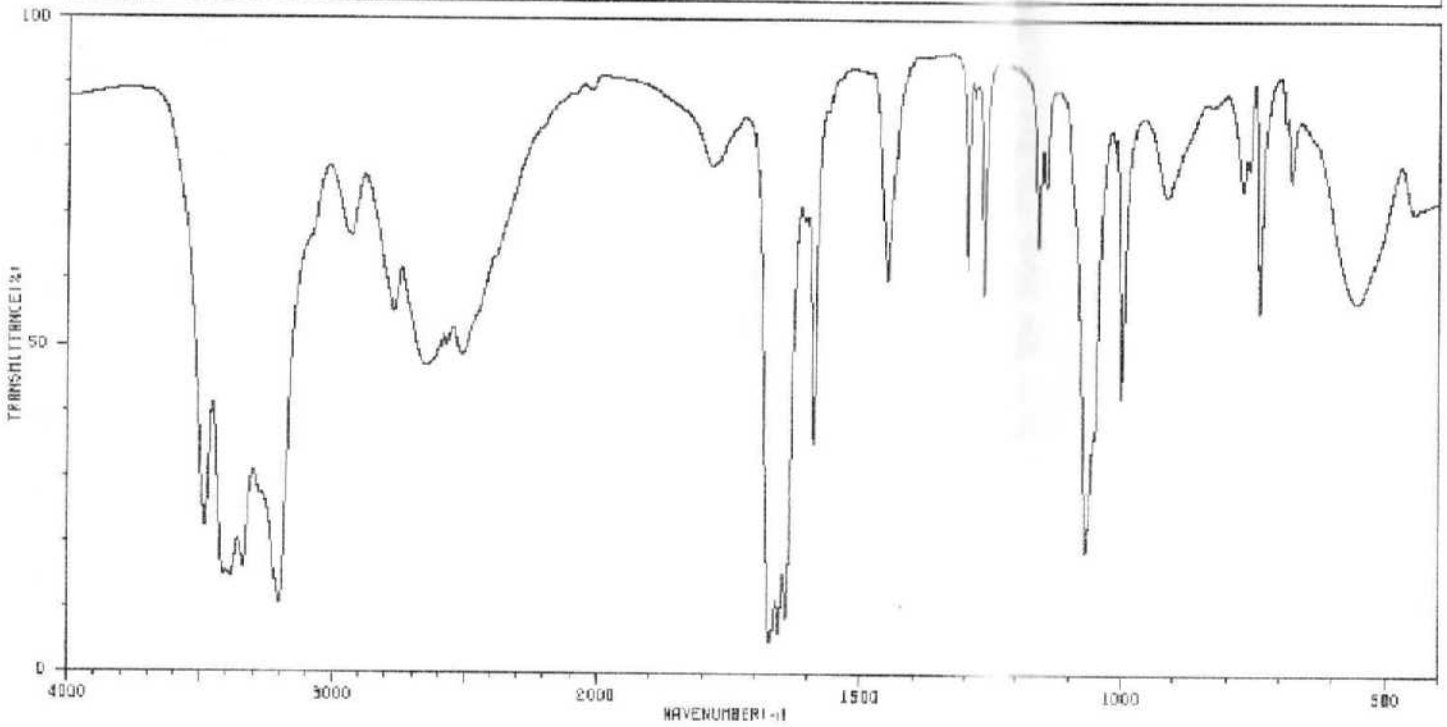


printed on 12/25/2018 02:20:25

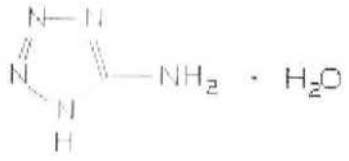
HIT-NO=1566	SCORE= ( )	SDBS-NO=1604	IR-NIDA-08268 : KBR DISC

1H-TETRAZOL-5-AMINE

$CH_3N_5{\cdot}H_2O$



3468	21	2846	48	1690	34	1068	18	890	81
3415	14	2573	49	1452	58	1019	77	679	72
3385	14	2510	47	1299	60	1001	41	554	55
3337	15	1782	74	1287	84	912	70		
3201	10	1671	4	1266	57	771	72		
2935	84	1658	5	1159	64	756	74		
2772	63	1641	9	1145	72	737	68		



SDBS Information

SDBS No.: 1604

Compound Name:
1H-tetrazol-5-amine

Molecular Formula: CH_3N_5

Molecular Weight: 85.1

CAS Registry No.:
4418-61-5

Spectral Code:
Mass :
^{13}C NMR : DMSO-d_6
IR : KBr disc
IR : nujol mull

Chemical Information:

Return to Search:
Return to Result:

URL for this Compound:
https://sdbs.db.aist.go.jp/sdbs/cgi-bin/landingpage?sdbsno=1604

External Information:
external link displays in a separate page

- Japan Chemical Information Link Center (in Japanese)
  - JST Nikkaji Web (in Japanese)

The was a very different approach to use an ionic salt, and it definitely produced a unique result. Methods now include for LC:
1. Acid-Base Chemistry
2. Redox Chemistry
3. Ionic salts Chemistry (Mg, Na, Ca – strong reducers)

Every method produces different results & isolates different Compounds.

Tube	ORP	pH	Notes
1-3	-80 mV	7.4	$MgSO_4$ - appear similar
to Tube 1-2	~~7.4~~	~~$MgSO_4$ - Clear~~	
1-4	+35 mV	7.4	$MgSO_4$ - Clear

Notice that the blue Cola does not elute from the Column, I am not sure why?
Get in the habit of testing all Eluents to make sure that no precipitates are formed prior to entry into the Column. NaCl passes the test also.

I have now switched to 50% Vinegar, 50% H_2O after $MgSO_4$ tabulation. I am getting significant reduction again.

1-5 -195 mV 7.1 50% Vinegar, Clear
The blue Color will still not elute from the Column. But 50% Vinegar may be pushing it downward.

Yes, it is definitely pushing the blue Color downward.

It would appear that the Acetic Acid / H_2O solution is neutralizing the blue color material.

But it must also be pushing it through as it is disappearing but Eluate is Clear.

This is all true. The blue color is now removed from the column.

Tube	ORP	pH	Notes
1-6	-185	6.1	Blue Color removed Vinegar / H_2O mix slight yellow color

Tubes 1-5 & 1-6 are the same Compound. It appear to be relatively pure due to the simplicity of the IR spectrum.

LC: Protein Tubes 1-5 & 1-6 Isolated Amine (Amine Salt likely)
Appears to be fairly pure.

CDB S

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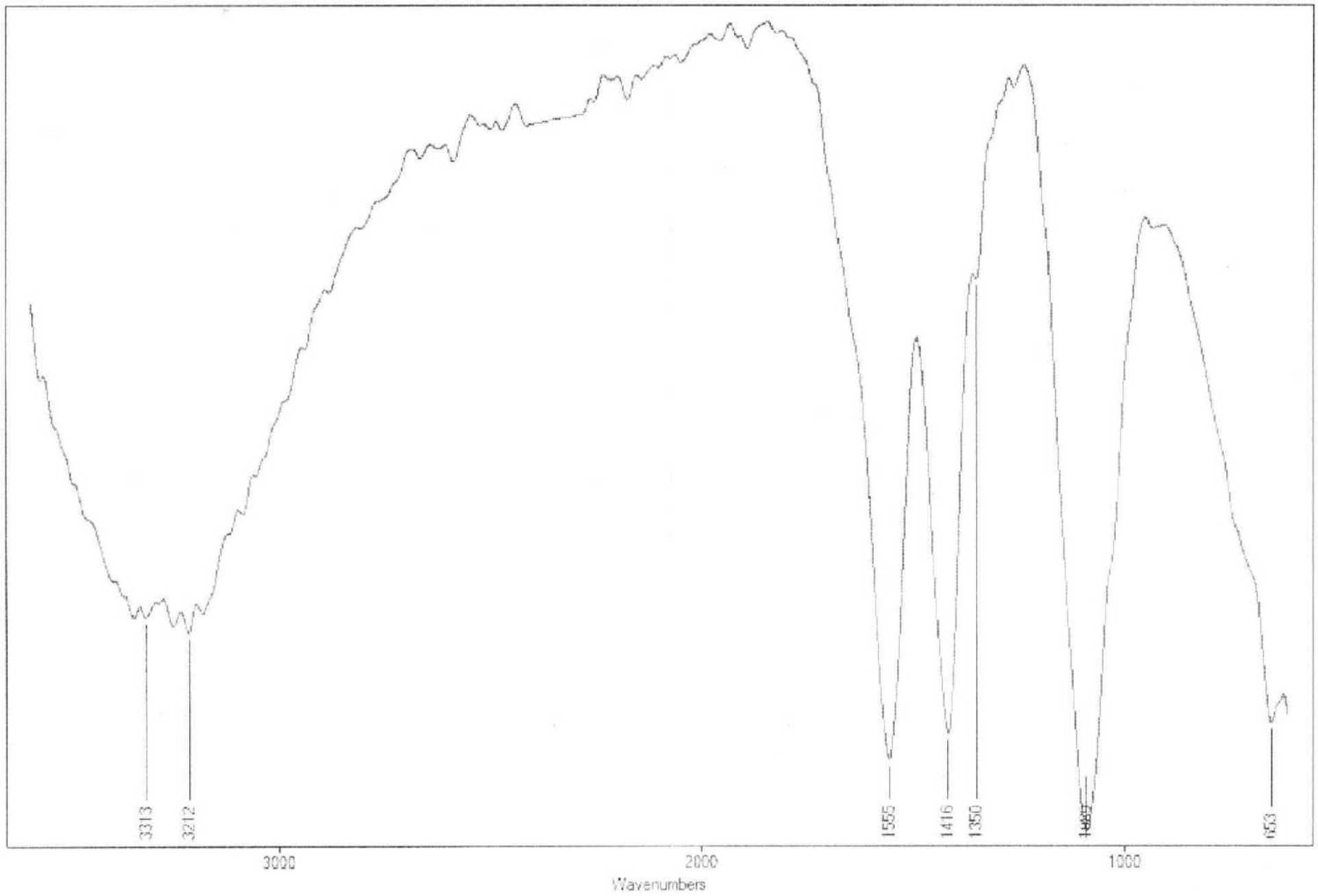
CDB Soluble Protein LC ATR Tube 1-6 OPR -185 pH 6-1 Dec 24 2018 - 14.spc: 12/25/2018 03:58:47 title



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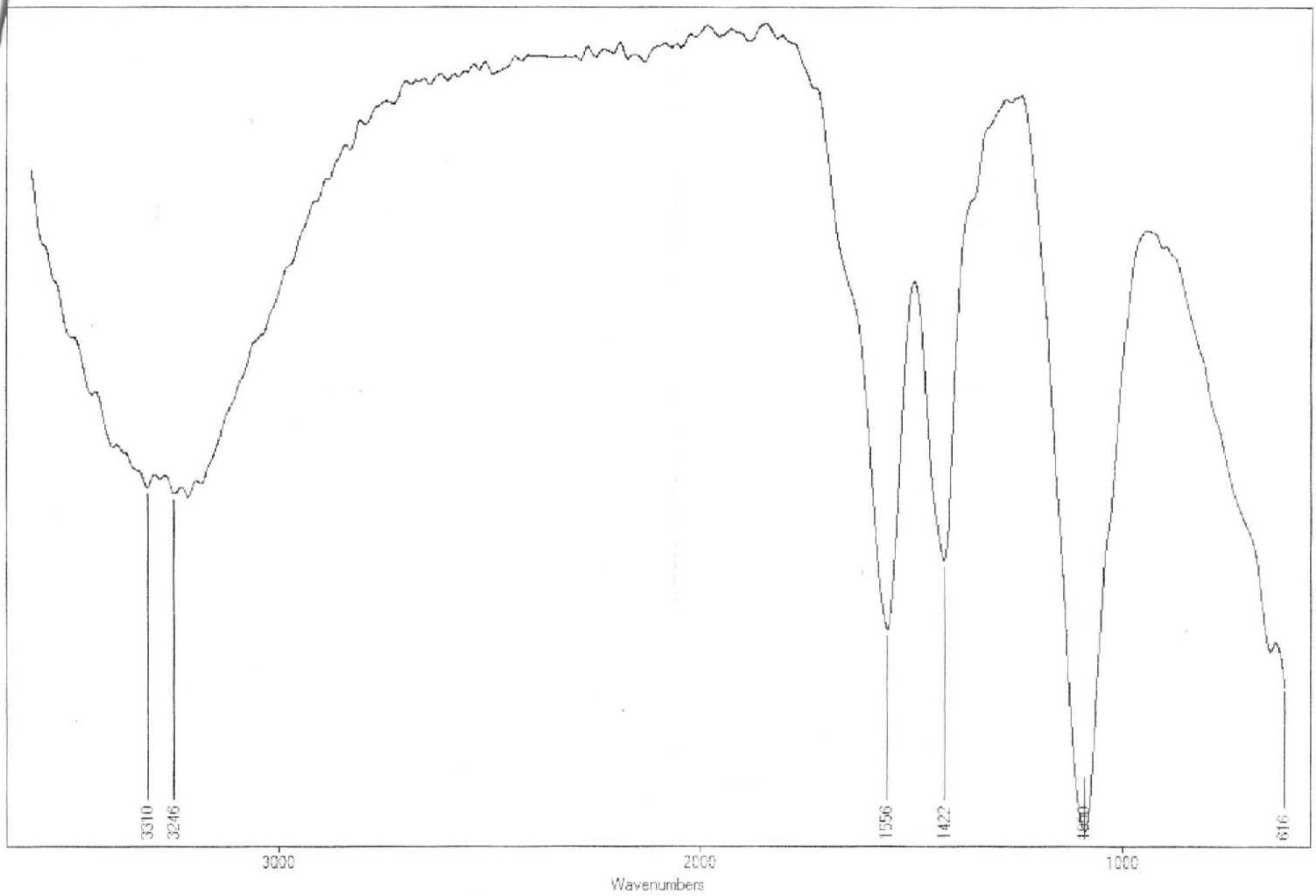
Protein LC ATR Tube 1-5 OPR -195 pH 7-7 Dec 24 2018 - 13.spc: Synthetic spectrum by averaging. See audit trail for details.



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Dec 26 2018

There are some very important discoveries that have taken place with respect to (w.r.t) to general chemistry of the soluble protein.

The chemistry of formation of the soluble protein is, or must be, remarkably similar to the process of fermentation of Kombucha.

This was discovered in the following manner

An article was read that discussed the potential hazards & toxicity of aldehydes that may exist w/in kombucha, a fermented drink.

Since the result of discussion remained vague, I decided to determine if aldehydes could be detected w/in both a homemade brew of kombucha and a commercial version.

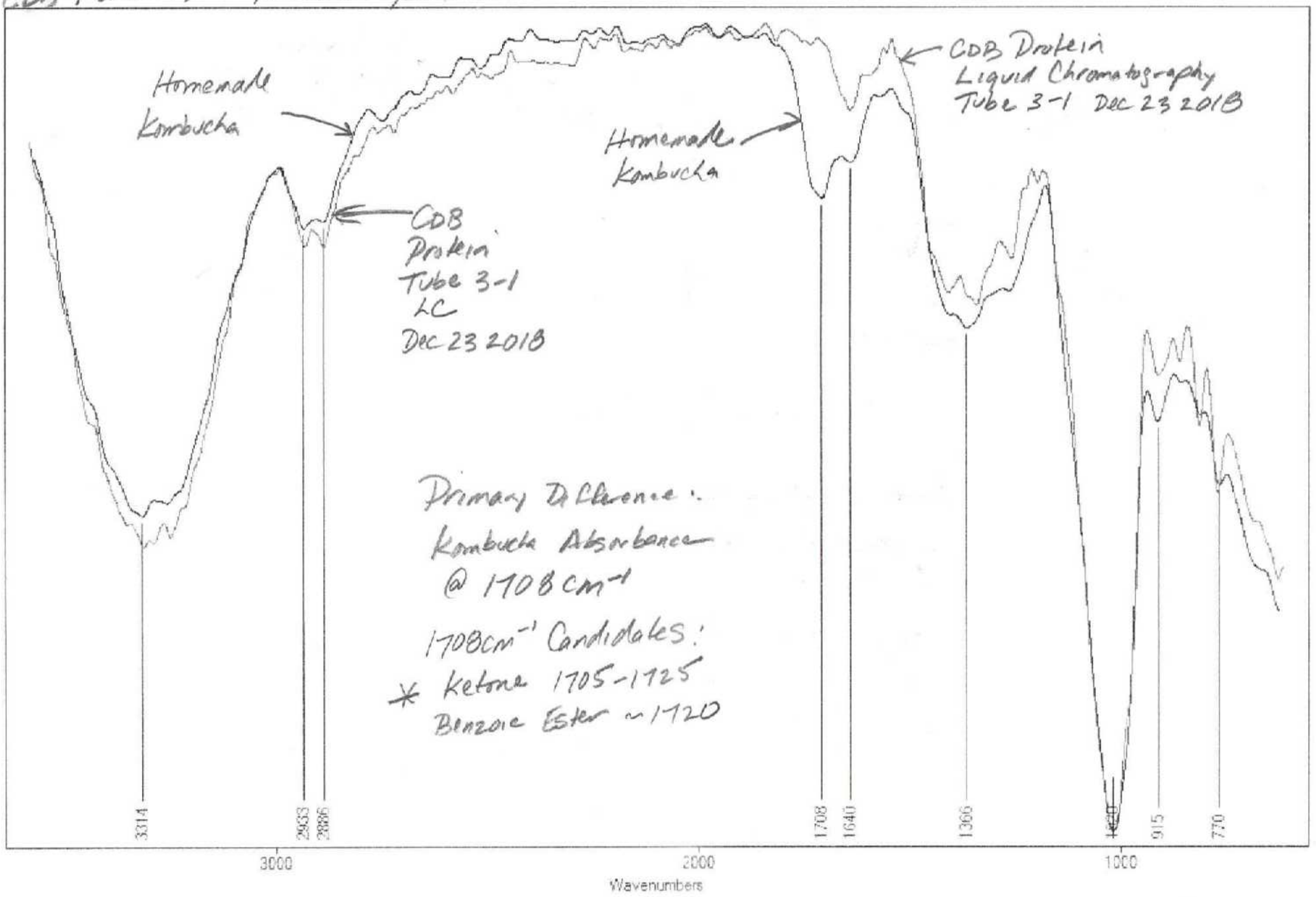
The answer is, by IR spectrometry, that the commercial version showed no aldehyde content and the homebrew kombucha showed a slight but detectable amount of aldehydes present.

The IR spectrum of the homebrew kombucha was then matched against the entire library of ~ 6000 spectra here.

The result a both fascinating and annoying.

Kombucha Homemade ATR Dec 25 2018 - 01.spc 12/25/2018 11:53:08 title

CDB Protein VS Homemade Kombucha



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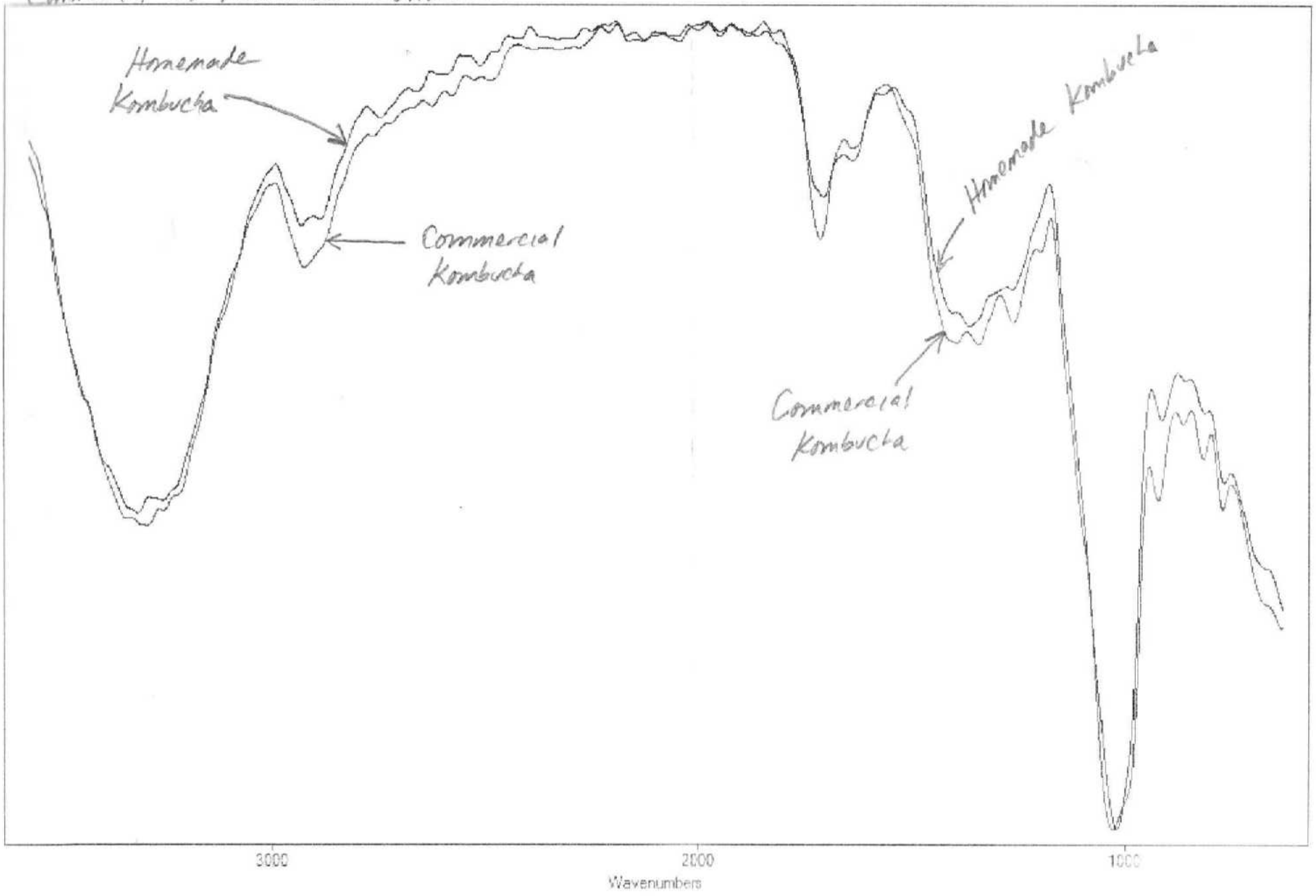
Homemade ATR Dec 25 2018 - 01.spc: 12/25/2018 11:53:06 title

Commercial vs Homemade Kombucha



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The "synchronicity", ie, divinely issued are certainly worth bringing to the forefront

* The closest match in the spectra is with:

* Liquid Chromatography Tube 3-1

* separated & collected by yours truly on Dec 24 2018, two days ago.

The IR spectra, attached, speak for themselves. They are remarkably identical, the primary variation is the COB absorbance 1708 cm⁻¹

* We must also conclude that the Chemistry of formation between the COB protein and the kombucha shall be found to be remarkably similar.

Aldehyde are apparently produced most commonly by yeast & fungi.

The things in the question of further evidence of confirmation of the Nomenclature "Class-Domain Bacteria"

which appears to remain as perfectly valid terminology.

Why? The originating microbe continues to satisfy all criteria of a bacteria. An extensive paper providing that list of bacteria has been written.

Now we also have extensive evidence & criteria that satisfy the Eukaryota domain. Visual & chemistry.

We also have evidence that they may well satisfy the criteria of the Archaea as well, due to their survivability under extremely hostile conditions.

There is a great deal to be learned now from the comparison of the fermented kombucha and the chemistry of the CDB soluble protein formation.

Analysis of the 1708 cm^{-1} peak will be important also, apparently a ketone.

This observation alone suggests a slight change of chemistry between the aldehyde & ketone groups could have a major impact upon the final product as well as the consequent health effects. Another rich topic of research exists here. If the chemistry of the CDB was altered to produce a ketone, it could alter or negate the aldehyde influence(?) - potentially a toxic one(?)

Another observation we have made:

The addition of bleach to the CDB protein causes a bold yellow color to be formed.

The addition of bleach to Kombucha does not produce the color, and the solution remains clear.

The reason for this is as follows: It has already been determined that the CDB protein contains a hydrated form of the Fe^{3+} ion.

This is important & consistent w/ the dominant role of iron understood for the microbe.

When bleach is added, $FeCl_3$ is made by replacement,

and $FeCl_3$ is the bright yellow color seen.

Accidentally, the bleach test ends up being a useful detector / indicator for the presence of the Fe^{3+} hydrated ion.

There is a lot of important study ahead, an important reference Chemistry process (ie Kombucha formation) now exists before us.

It is true that some valuable LC (Liquid Chromatography) work has been done here and numerous & various compounds extracted or related to the CDB soluble protein have now been isolated and separated. The reactions of the protein w/ the eluent used are important to consider w/ their flow products that have formed.

Back to some titration examinations.

Bleach + Vinegar → Not good - Cl_2 gas!

NaCl + Bleach → highly dangerous

Ammonia + Bleach → highly dangerous

$MgSO_4$ + NaOH → precipitate $Mg(OH)_2$

H_2SO_4 + bleach → highly dangerous → Cl_2

Bleach is NaClO - sodium hypochlorite

hypochlorite ion is ClO^-

$$\overset{+2\ -2}{MgSO_4} + \overset{+1\ -1}{NaClO} \rightarrow Mg^{+2} + SO_4^{-2} + Na^{-1} + ClO^-$$

(It definitely dissolves)

$MgCl_2$ is Magnesium Chloride (No redox apparent)

Sodium hypochlorite is Chlorine gas dissolved in sodium hydroxide

— lots of flags there.

However

$$\overset{+2\ -2}{MgSO_4} + \overset{+1\ -1}{HCl} \rightarrow \overset{+2\ -1}{MgCl_2} + H_2 + \overset{-2}{SO_4}$$

Mg

replacement, not redox

$MgSO_4$ ok

$$\overset{+2\ -2}{Mg}\overset{+6\ -6}{SO_4} + \overset{0}{Fe_2}\overset{+2\ -2}{(SO_4)_3} \rightarrow Fe + MgSO_4$$

Mg^0

Mg oxidized
Fe reduced

but this is magnesium metal, not ionic form

Mg^{+2} cannot be oxidized further because it is already inherently stable.

This has to do w/ the electron configuration

Mg: $1s^2 2s^2 2p^6 3s^2$

or $[Ne]\ 3s^2$

Mg^{+2} has the configuration $[Ne]$ which is stable

Try to make Mg^{+3} and you get:

hypothetical Mg^{+3}: $1s^2 2s^2 2p^5$

Which is the same as Fluorine except for a nucleus w/ three more protons.

Mg^{+2} can not be oxidized further.

So obviously the electron configuration is important toward understanding the likelihood of redox reaction.

Dec 26 2018

To me, there seems to be a distinct advantage in performing @ least some titrations using mass percent instead of molarity.

It means that I do not need to know what something is made of or its chemical composition and I can still regulate it in some form (i.e., mass percent) with something that I do know.

Also I can determine density of solution by weight / volume and can titrate using the change in mass instead of counting drops.

Let's try this:

Mass of graduated cylinder (100 ml) = 127.0 gms
Add 3.5 gms of NaOH
Bring volume to 100 ml.
230 gms total mass

Therefore: 230 gms - 127 gms (cylinder) = 103 gms
(total mass) NaOH + H_2O

Our mass / volume % = $\frac{3.5 \text{ gms}}{103 \text{ gms } (H_2O + NaOH)}$ = 3.4% NaOH by Vol

our mass percent
Concentration is $\frac{3.5 \text{ gms NaOH}}{99.5 \text{ gms } H_2O}$ = 3.5% mass percent.

MW of NaOH = $\frac{40.0 \text{ gms}}{\text{mol.}}$ $\frac{40.0 \text{ gms}}{100 \text{ ml}}$ = $\frac{4.0 \text{ gms}}{100 \text{ ml}}$ = 1M

~~103 gms~~ ~~ml~~

We have a $\frac{3.5 \text{ gms}}{4.0 \text{ gm}}$ = .875M NaOH Solution.

↑ Final volume of water + solute

So understand that we placed

3.5 gms NaOH The w/v solution.
100 ml (final volume)

1M solution = 40.0 gms / 1000 ml = x / 100 ml = 4.0 gms

However, we have 3.5 gms, therefore we have a

3.5 gms / 4.0 gms = 0.875 M NaOH solution final.

Our mass/final volume ratio is 3.5 gms / 100 ml = 3.5% mass to volume ratio.

↑ final volume

Dec 27 2018

Stoichometric & pH computation software is now in place:
1. BATE – ChemBuddy – Titration software
2. EBAS – ChemBuddy – Stoichometric software
3. CHEMX – Chemistry Toolbox / Utility software
   (Latter in place for many years)
4. REDOX software – Half Reactions

Urea Composition: We could construct a Compound Internate here.

1. ~ 93.5% H_2O
2. 6.07 gm/liter C
3. 8.12 gm/liter N
4. 8.25 gm/liter O
5. 1.51 gm/liter H

About 2.5% of urine is urea & uric acid. This is about 96% total therefore.

—

Note that for many compounds (ie, all those w/a density close to water) that molarity and molality are actually very close to one another. The reason is because

molarity = moles / liter
molality = moles / kg of solvent

and since w/ water, 1 liter = 1 kg, the results in many solutions w/ molality $\approx$ molarity.

Now you must separate molality & osmolality!

Osmolality Definition:

1. The total number of "solute particles"
   ——————————
   kg of solvent

Let's look @ definitions a little further:

McGraw-Hill book:

The molality of an ideal solution of a non-dissociating substance that exerts the same osmotic pressure as the solution being considered.

(Have fun w/ that one)

Oxford had no definition.
Chemmac had no definition.

Definitions are quite varied, but basically you are dealing w/ a measurement of all dissolved particles (~~not~~ both ionic and non-ionic to me) that are in solution, and that the freezing point depression responds to all of them as they are colligative in nature.
Colligative properties depend upon the number of particle present and NOT the chemical species

Molarity depends totally upon the chemical species(s) that
are present as the molecular weight of the compound is required to determine the molarity.

There is the fundamental difference between molarity and molality.

Molarity: You must know the chemical species involved

Molality: You may or may not know something about the chemical species(s) involved, but you do not have to. You only need to know the NUMBER OF PARTICLES present, which can be determined by a fancy instrument that measures the freezing point depression.

If you do know the species, and the density of the species in solution is close to that of water, then the molarity and the molality will be close to one another, and this will often be the case.

Example:
A 5% NaCl solution has a molarity of 0.856 M
A 5% NaCl solution has a molality of 0.870 m

This will end up being quite common, and equatable to some degree even with a complex compound such as urine, made up mostly of water (96%) & urea (~2.5%).

There will be a lot of basic study that will take place here.

* Where is this all headed? It is toward a better understanding of the nature of the proteins that have been isolated from the COB.

Fundamental knowledge about the nature of the protein and its energy transfer characteristics are important. This will be and is beneficial toward developing strategies to mitigate the effects of these proteins, which are now rather conclusively known to be toxic to some degree.

The focus will be on the soluble protein, as this will be and is the easiest to work with now in terms of chemical reaction study.

An emphasis will be based upon:

1. Acid - base chemistry
2. Redox Chemistry
3. Electrochemistry - a whole field in itself
4. Titration
5. Stoichiometry

The likelihood of a redox reaction occurring is another fundamental to have knowledge of!

Basically if

Voltage of Reduction Half Reaction	+	Voltage of Oxidation Half Reaction	>> 0 Then the reaction is much more likely to occur
(Strong oxidizers will have a positive voltage here)		Strong reducers will have a high positive value when written as an oxidation half reaction	
Acid added to Iodine solution to see what happens		They will have a low negative number if the reducers are written as Reduction Half reaction	

If both reduction and oxidation half reactions are listed as ~~HALF~~ REDUCTION half reaction, then

Voltage of Reduction Half Reaction	−	Voltage of Oxidation Half Reaction WRITTEN AS A REDUCTION HALF REACTION	>> 0 Then the reaction is likely to occur

Example

Bleach + Iodine ⇒ A Definite Redox Reaction

HClO + I^- ⇒ +1.14V

+1.63V + (−.49) =

The REDOX software is especially useful for helping to evaluate these interactions

In the REDOX software, all potentials are listed in the "Reduction Half Reaction" format, therefore with redox

We use the relation:

Voltage of Oxidant − Voltage of Reductant (written in the Reduction Half Reaction format) ≥ 0

~~HCl~~
HClO
1.63V − (Cu^{+} (+.15)) = +1.48V ≥ 0

So the reaction is expected to occur

There is definitely a reaction, a precipitate forms.

So bleach added to iodine and $CuSO_4$ (Provoline) works produces definite redox reactions.

There are great examples to work with in potentiometry & titration.

Also, pH plays a significant role in the likelihood of a redox reaction occurring so I consider it imperative that you consider this in the evaluation and identification of the particular redox reaction that is occurring.

Fortunately, the REDOX software includes this feature.

It is already known and understood that the soluble protein has the hydrated Fe^{+3} iron form in it, and that it is strongly acidic. This is a good start.

I have a test titration example set up.

use 5% acetic acid (~vinegar) – ~~100~~ 50 ml M=.8326 M/l

NaOH: 3.33gms in 100 ml M=.8326 M/l

make · 6.66gms in 100 ml to read equiv. pt @ 50 ml.

Our reaction proposed is

$$CH_3COOH_{(aq)} + OH^- \rightarrow CH_3COO^- + H_2O$$

The stoichometry shown should reach the equivalence point w/ a pH of 9.16

Notice the CHEMIX concentration module is superior to Chembuddy EBAS as it provides computation of molality also which incorporates density information within the concentration expression. This can be helpful, especially when dealing w/ osmometry applications.

Now the next question is, what type of titration to actually perform?

1. OCP ~~ORP~~ give graphic information. But does OCP always record acid-base chemistry and if so how?

2. ORP seems especially general & helpful. But does it too accurately visualize acid base titration?

3. pH meter - the obvious choice but no graphics can be incorporated w/ a regression

ORP electrodes often use a Ag/AgCl saturated KCl reference electrode

Our OCP electrodes are graphite -

Let's try it.

Note on Formal Charge determination.

The simple and fast method is:

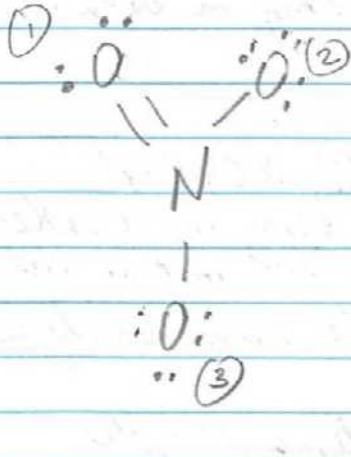
Formal charge = Should − Has

(Normal expected no. of valence electrons the atom w/in the molecule should have) — How many electrons the atom within the molecule actually has. DIRECTLY ATTACHED, NOT SHARED

Formal charge is very important to understand what is happening INSIDE of a polyatomic ion.

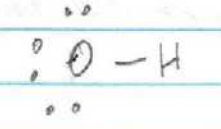
$[NO_3]^{-1}$ $[OH]^{-1}$ DOES NOT tell you what is happening inside the ion.

The above method of formal charge determination does



N: 5 − 4 = +1
① O: 6 − 6 = 0
② O: 6 − 7 = −1
③ O: 6 − 7 = −1
Σ = −1

$[NO_3]^{-1}$



H: 1 − 1 = 0
O: 6 − 7 = −1
Σ = −1

$[OH]^{-1}$

Functional group study (Leah 4 sci)

Nitrogen bound to:

Amines	Primary	Secondary	Tertiary	Quaternary
the "N" is primary	1 "R"	2 "Rs"	3 "Rs"	4 "Rs" (with a charge)

Ether (Either one can be an "R")

R—O—R

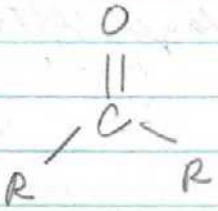
Alcohol (COH) is within

Thiol "ol" means alcohol

OH, erase the Oxygen & replace w/ sulfur for SH is thiol

Carbonyl

Ketone (Doesn't want to "be alone"

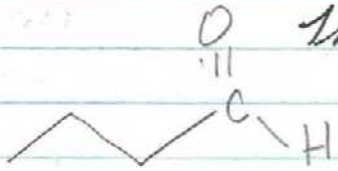


Wants something on both sides

Carbonyl

Aldehydes I see it as "~~being~~ a carbonyl on the side" with a H (instead of OH)

This is all in the name or a rhyme,



hyde rhymes w/ side and "H" is in the name

Carboxylic Acid COOH $R-C(=O)-OH$

Ester → a R group swapped out for H on a carboxylic acid.
An ester is a carboxylic acid group where the H has been swapped out w/ an R
ie COOH → goes to → COOR

also is a carboxylic acid derivative

Remember ether was "either R" $R-O-R$

Amide - An amide "looks like a carboxylic acid but sounds like an amine"

Carbonyl ↑ d also means a double bond

$R-C(=O)-NH_2$

("Sounds like an amine, looks like an amine," but the "d" means a double bond - carbonyl)
It is indeed a "carboxylic acid derivative")

(does not have to be a primary amine)

Nitrile: $R-C\equiv N$ Nitrile has a $C\equiv N$ bond.

Phenol (not actually a functional group) → Phenyl

"ol" meant an alcohol

"yl" means a R group as a substituent

Phen means benzene ring





Onward to the titration. I will use the OCP method to make things interesting.
I will also use mass of the titrant in addition to counting drops as a reference

Mass of weigh boat = 30.01 gms
Mass w/ 10 ml syringe (dry) = 37.80 gms
Mass of syringe = 7.79 gms
Vinegar + NaOH Titration Δ +

t(sec)	Drops or Volume	OCP	Syringe Mass	Notes
				It is taking some time for the OCP to stabilize in the vinegar analyte.
			Ref = 15.71 gms	
1160	~~10~~ 0	31 mV	~~15.15~~	
1200	10	27	15.15	OCP decline
1360	20	25	14.56	
1420	40	24	13.35	
1500	70	18	11.57	sharper decline
1605	100	7	9.79	
1780	150	+1	Ref = 19.94 gms	
1850	200	-12	17.01	
1900	220	-26	15.81	
2130	250	-49	14.02	
2250	300	-58	11.02	
2380	350	-61	8.17	
			Ref 18.68	Bottoming out now
2500	350	-61		Starting to rise.

It has clearly shifted to an alkaline state based upon pH paper test.

We have, therefore, easily crossed the equivalency point. The min appear to have been reached @ 2388 secs.

So what exactly was reached @ 2388 secs?
Let's titrate backwards and see how much vinegar is required to swing the pH backwards.

The current pH is ~ 11.9
The equivalency pH is stated to be ~ 9.2

So obviously we have a problem here. Intuitively it is known that the NaOH solution is far far too strong, but the computations do not coincide. Why?

You would need to make the NaOH solution ~ 0.1 of this to even have a chance.

~~15.11 gms~~

	Drops	pH
	0	11.9
	50	12.0
+	2ml	12.1
+	4ml	12.1
+	6ml	
+	6ml	
+	6ml	
+	15ml	
+	30ml	11.5
+	30ml	6.5 ← equivalency point.
Σ=	100ml	

This work also shows that the OCP method can not be used (as practiced) for thin acid - base Chemistry.

Recommendations for further trials:

1. Construct a NaOH solution 1/10 M of the current, ie, 6.66 gms will go to 0.66 gms/100 ml.

2. Perform the titration again, this time use the pH meter.

3. Examine the calculations in light of what is found through observation.

It makes you wonder what OCP is actually measuring here, considering that we dynamically have a
   1. sharp slope drop
   2. a maximum potential reached

but the interpretation of these is a different matter. You will need to couple a pH meter w/ the OCP measurements to help sort them out.

The next titration. Time not needed 50
Now we have 0.7 gms NaOH in 100 ml H_2O
100 ~~50~~ ml vinegar
Will use wgt instead of drops, and pH instead

	Wgt	Δ mass (gms)	pH	Notes
Ref.	17.14 gms		3.90	
	13.35		4.09	Much easier by mass.
	10.09	7.05	4.28	Can watch melt instead of drops
Ref.	16.45			
	9.91	6.54		Consider heating it up to dry out.
Ref	19.02			
	9.15	9.87	4.90	NaOH is highly hygroscopic and also absorbs CO_2
Ref	18.62			
	9.79	8.83		
Ref	17.76			It cannot be weighted accurately. ~68 grams vs 40
	8.97	8.81	5.37	
Ref	18.90			
	9.15	9.75		
Ref	17.89			Reduce eluent volume by $40/66 \cong 0.59$
	9.15	8.74	6.00	
Ref	18.25			
	9.68	8.57	6.65	
Ref ~	18.28 (missed it)			This is the region.
	13.70	4.58	10.15	Just barely enough NaOH.

$\Sigma = 72.74$ gms
$\cong 72.74$ ml

only anticipated to use ~50 ml.

You could easily double the NaOH concentration. Use 1.4 gms.

We have noticed a change in the pH software Calculations.

It appears that a corrected mass of NaOH to reach the equivalence point is 1.665 gms, instead of 0.666 gms. No idea where that error came from. I reset the data holding the molarity constant.

This introduces a titrant volume error factor of $\frac{1.665}{.666} = 2.50$

The suggestion we would have added

$\frac{72.74 \text{ ml}}{2.50} = 29.1 \text{ ml}$ of titrant

but our calculations anticipate that 50 ml will be used. Why the difference.

We expect that $\left(\frac{50 \text{ ml}}{72.74 \text{ ml}}\right)^{-1}(0.1 \text{ gms}) = 1.029 \text{ gms NaOH}$

be used, not 1.66 gms.

No idea why of the discrepancy.
Hygroscopicity does not seem to be an issue here.

Dec 28, 2018 Quantum Numbers

1, 3, 5, 7 # orbital
2 6 10 14 # electrons
s p d f energy

this is the take away along with the shapes

Principal No. Angular Momentum No. Magnetic Quantum Number

Spin $(+\frac{1}{2}, -\frac{1}{2})$ Factor of 2

Quantum Numbers

Defines:
1. Sublevel #
2. shape

n $l = \emptyset$ to $n-1$ $m_l = -l$ to $+l$

ENERGY LEVEL	SUBLEVELS	# OF ORBITALS	# of e^-	Total e^-
1	s ($l=\emptyset$) 0	1 ($m_l=0$) (the s orbital) s	2	$2n^2 = 2$
2	s, p ($l=0,1$) 0 1	s, −1, 0, 1 1, 3 = 4 s, p	(2+6) 8	$2n^2 = 8$
3	s, p, d ($l=\emptyset,1,2$) 0 1 2	s, −1, 0, 1, −2, −1, 0, 1, 2 1, 3, 5 = 9 s, p, d	(2+6+10) 18	$2n^2 = 18$
4	s, p, d, f ($l=\emptyset,1,2,3$) 0 1 2 3	0 1 2 3 s, p, d, f 1, 3, 5, 7 = 16	(2+6+10+14) 32	$2n^2 = 32$

12:59 / 13:27

Science

The s, p, and d Orbitals

n	# orbitals	e^-
1	1	1→2
2	3	3→6
3	5	5→10
4	7	7→14

(a.) s Orbital

(b.) p Orbitals

(c.) d Orbitals

Figure 3.1 The s, p, and d Orbitals

Apparently, quite a bit harder to visualize the "f" orbitals!

Furthermore, the progression 1, 3, 5, 7 orbitals is the most practical to remember s p d f

The progression of 2, 6, 10, 14 # electrons is the most useful

The purpose & value of the quantum numbers is to be able to tell WHERE the electrons are going to be. Where is the end goal here!

electrons = 2x # of orbitals

More properly visualize WHERE the electrons are. Total No. of electrons up to & including each energy level is $2n^2$

Synthesizing the quantum nos. for the practical take away is important here.

Three progressions end up being practical to recall

energy level	s	p	d	f	
	↕	↕	↕	↕	
no. of orbitals	1	3	5	7	
	↕	↕	↕	↕	
no. of electrons	2	6	10	14	$\Sigma = 32$
	one sphere	Three sets of a two lobed region	5 sets of variable lobe shapes	7 sets of even more complex lobes	

This is occurring for each energy level

Understanding the sequence and method by which these orbitals fill up is the next stage of understanding here.

The periodic chart is the window to this understanding.

Aufbau principle & Hund's rule express this sequence.
(From P orbitals upward)
Each orbital accepts one electron before the other orbitals begin accepting the second electron.

Ok, What is needed now is to develop a visual construct of these electron configurations as they coincide w/ the layout of the periodic table.

Time for some mental & visual analysis -

Ok, I see the problem w/ respect to (w.r.t) the S, p & d blocks of the periodic table.

S's & p's are at the same energy level. The just mean that the shapes of the energy levels are different.

D block is reduced in energy level by one level so it is enumerated before the subsequent S & p blocks.

You should now be able to visualize the electron configuration of any of the lower elements, up to element No. 56 (Barium).

Platinum & gold will get into the f blocks, which are reduced even one more layer down than the D block is.

Ok, I have these pictures in place:
1. Orbital shapes & positions & energy levels
2. Electron configurations visualized along w/ Hund's rule

Next we will work on hybridization & VESPR.

Determination of stearic number will determine the hybridization.

This is only for stearic number not formal charge

— = ≡ all count as 1

∘∘ lone pairs count as 1

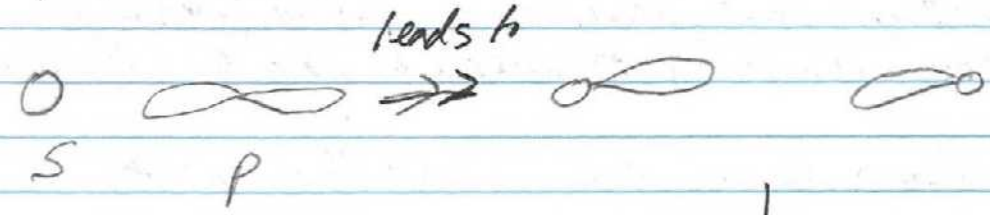
Sum the count

	Hybridization	Stearic Count
2 bonds is ~~combined~~	sp	2
3 bonds	sp^2	3
4 bonds	sp^3	4

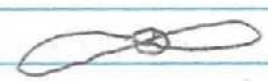
and then you can visualize now. Unused p orbitals can lead to pi bonds.

Understanding the geometry of hybridization allows you to understand the geometry of the molecule

sp looks like this:



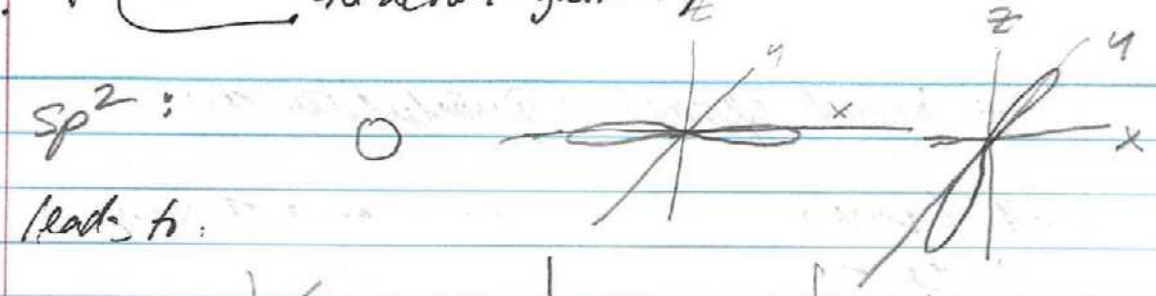
leads to

a linear arrangement 

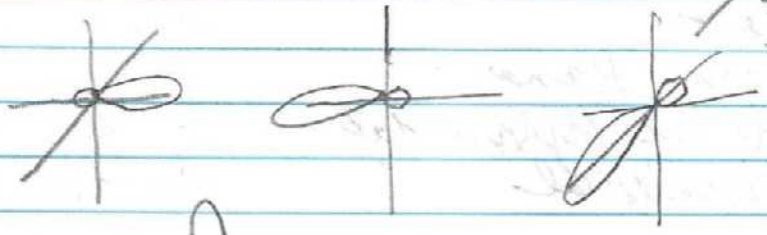
with two unused p orbitals.

!! (You must consider lone pairs in the Lewis Structure to determine the actual geometry) !!

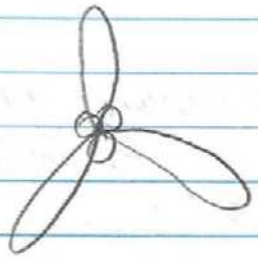
sp^2 :



leads to:



leads to



Trigonal planar 120°

And we have an unused p orbital

When there are no lone pairs!

sp^3 leads to a pyramidal arrangement., angle = 109.5° w/ no unused p orbital.

1. Draw Lewis Structure Determine the no. of lone pairs
2. Determine hybridization & keep track of lone pairs

The lone pairs are critical to understanding the geometry.

Lone pairs "take up a lot of space"

You must consider lone pairs & their effect upon geometry.

Lone pairs \ Steric Number	2	3	4
0	linear	trigonal planar	tetrahedral
1	linear	bent	trigonal pyramidal
2	N/A	linear	bent

Visualize this and apply vs memorize chart, it.

Our actual geometric possibilities are:

1. Linear — Top View
2. Bent — Top View
3. Trigonal Planar — Top View
4. Trigonal Pyramidal — Front View
5. Tetrahedral — Front View

ie, how many ways can you arrange geometrically symmetrical 2, 3, or 4 pts in space.
Think of the balloons.

Yes, use reasoning to establish the geometry.

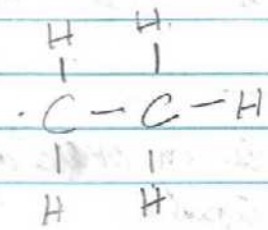
Then gets us through:

1. Orbitals, Energy Level's
2. Electronic Configuration
3. Hybridization
4. Molecular Geometry (VESPR)

Notice that p orbitals (eg 2p) will be a little higher energy than 2s orbitals.
There is no need that an sp orbital, it starts out w/ 2p.

Formal Charge can be used to determine (or assist greatly) in determing the Lewis Structure.

$[C_2H_5]^+$



It is correct

Formal Charge:

Wants − Has = +1

Valence Electrons

Electrons Directly attached to atom, NOT BONDS!

C_1: 4 − 3 = 1

C_2: 4 − 4 = 0

Σ = +1 Formal Charge Matches

Lewis Structure & Formal Charge determination go well together.

C wants 4 bonds
N wants 3 bonds
O wants 2 bonds
Halogens ~~[illegible]~~ usually only has one bond
H wants 1 bond.

Covalent bonding usually occurs between 2 non metals

"FONCIBrISCH" is a compact & comprehensive way to establish a ranking of electronegativity

most electronegative

least electronegative

The most looming question is how the geometry of a molecule affects its reactivity.

—

A "head on overlap" of two orbitals is a sigma bond



(does not have to be S, could be p also)

S orbital orbital

Helpful to understand that the electrons shared in the bond do NOT have to be equally distributed w/in the S orbitals.

It is quite possible that both electrons could be located in the right side S orbital, for example. This induces polarity into the molecule and therefore additional bonding forces.

→ The answer to the question is the same as it is for proteins:

"Structure Determines Function"

and I might add, reactivity & operation ...

It is always @ the core of understanding how one molecule interacts w/ another.

Shape determines function:

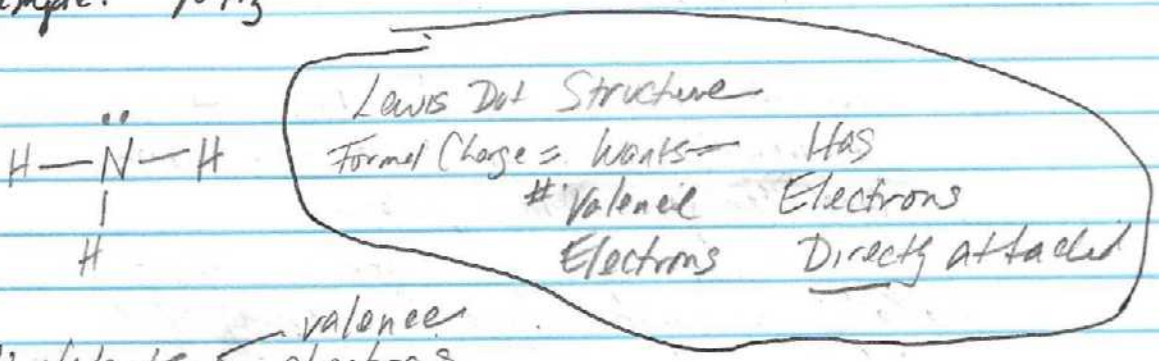
Cookie sheet vs a kettle:
Can you boil water in a cookie sheet (efficiently?)
Can you bake cookies in a saucepan (efficiently)?

That pretty much answers the question, doesn't it.

Do a great first practice problem is:
How does $[Fe(OH)_6]^{3+}$ react w/. NaHClO ??

Is geometry important here? What is the geometry?

Example: NH_3



N: Wants 5 valence electrons
Has Directly attached: 5 $5-5=0$
Formal Charge on NH_3 is 0.

Now Hybridization: / // /// or •• Counts as 1 Steric No

Steric No = 4.. Should be sp^3 hybridization.
Trigonal pyramidal.
Lone pair pushes the three legs down ~~symmetrically~~ symmetrically.

FONCl Br ISH - highly polar bonds.

Formal Charge Determination
Uses Valence Electrons — Electrons Directly Attached to Atom
WANTS HAS

and

Hybridization determination
Uses Stearic Number: / // /// or ••
2=sp 3=sp^2 4=sp^3
are two entirely different processes.

—

Two ways:
1. Draw in no of expected bonds w/ particular atom
2. Fill out according to octet rule and hydrogen (2) electrons

or
Construct valence electrons for each atom and then join them together.

Method 1 is quicker but less insightful
Method 2 is slower, tedious, but ultimately more insightful.

Lewis Structure + Hybridization → allows for Geometric Interpretation
(includes available one pairs by definition)

Steps Toward Geometric Understanding of a Molecule:

Stearic, w.r.t. Chemistry & VSEPR (repulsion) theory is equal to the number of atoms bonded pts / no pairs.

5. double bonds do indeed only count as 1, yes: / // /// or •• count as 1.

* Steps Toward Geometric Understanding:

1. Lewis Structure & Formal Charge go together

Formal Charge = Wants (# of Valence Electrons wanted) − Has # of Electrons Directly attached to the atom

2. Stearic Number is an intermediate to determine Hybridization

Stearic No: / // /// or •• Counts as 1

Stearic No	Hybridization
2	sp
3	sp^2
4	sp^3

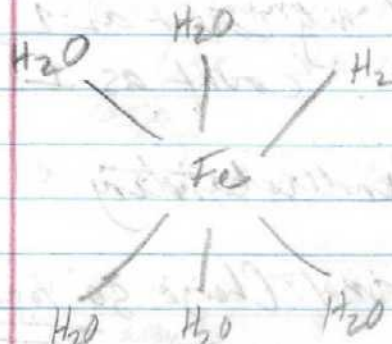
3. Hybridization coupled with Lone Pairs (determined from Lewis Structure) Available
Determine the Geometry

4. FONCl BrISH determine the Polarity
(i.e, electronegativity.

5. Avogadro will help to visualize this process.

$[Fe(H_2O)_6]^{3+}$? $\rightleftharpoons$ NaClHO

leads to $[FeCl_4]^{-1}$



$[FeCl_4]^{-1}$

is yellow tetrahedral

$FeCl_3$ reacts w/ chloride salts to give $[FeCl_4]^{-1}$

This looks like us

$[Fe(H_2O)_6]^{3+}_{(aq)} + 4Cl^-_{(aq)} \rightarrow [FeCl_4]^{-1} + 2H_2O$

from docbrown.info

∴ tetrachloroferrate $[FeCl_4]^{-1}$

Jan
Dec 04 2019 - Quartzsite, AZ! Titration Studies

On the road for the winter. Will progress through some fundamental titration for a while and also work out field setups.

First is acid-base: Vinegar + Ammonia.

Vinegar: 4 ml acetic acid (5%) in 20 ml H_2O = 20%
i.e. (0.20)(0.50) solution = 0.10% = 10% solution

Ammonia: 2 ml Ammonia in 20 ml H_2O.

pH measured @ +220 mV with pH meter electrode.
I will count drops this time. Weight next time. Delay 30 sec

Drops	mV	pH	Drops	mV	pH	Drops	mV	pH
0	+160	3.5	480	205		+8 ml	+190	
10	+160		530	+205	4.4	+8 ml	+180	
20	+180		580		5.1	+8 ml	+165	
30	+180		630	+220		+4 ml		
50	+180		680	+200		+8 ml	160	
80	+190		730	+210	4.2	+8 ml	+150	
120	+190		780	+220		+5.5	+145	
170	+195		830	205				
230	+190		+ 8 ml	203				
280	+230		Doubled NH_3 Strength					
+290	+220		+ 8 ml	+205				
+300	+220		+ 8 ml	+205				
+330	+210		+6 ml					
+380	+215	4.0	Tripled NH_3					
+430	+210		+ 8 ml	~~+210~~ 210 +180				

Ok, this is the end of this trial. Interesting.

Well, your first run is only useful for apparatus setup. The electrode used was incorrect, EC instead of pH. All electrodes have now been prepared and labeled
Conductivity (EC)
pH
ORP.

Let's run w/ vinegar & baking soda.
Titrant Vessel: 127.22 gms.
w/ Baking soda 136.45 Δ = 9.23 gms
w/ H_2O added 284.82 Δ_{vessel} = 157.60* gms

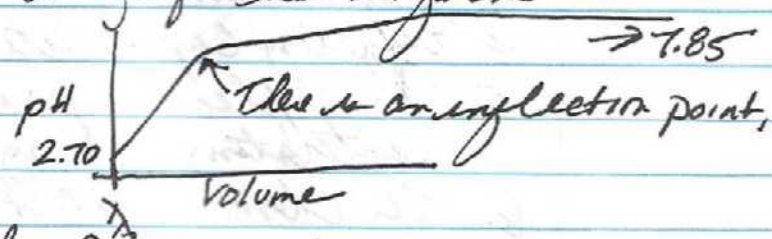
% Volume = 9.23 gms / 157.60 gms = 5.86 %
pH = 7.85

Analyte: 8 ml vinegar in 40 ml H_2O.
pH ≅ 2.75

Mass	gms ΔV	pH
* 18.03 ref	0	2.70
17.23	0.80	3.40
16.12	1.91	3.80
14.54	3.49	4.14
11.88	6.15	4.58
9.63	8.40	5.00
* 17.63 ref	8.40+	
15.87	10.16	5.40
14.44	11.59	5.75
13.51	12.52	5.95
12.72	13.31	6.10
11.87	14.16	6.26
11.02	15.01	6.41
9.55	16.48	6.58
* 18.71 ref	16.48+	
16.03	19.16	6.79
13.36	21.83	6.96
10.89	24.30	7.10
8.66	26.53	7.20
* 17.81 ref	26.53+	
14.03	30.31	7.33
9.42	34.92	7.44
* 18.06 ref	34.92+	
7.81	45.17	7.56
* 19.03 ref	45.17+	
7.87	56.33	7.64

Mass method is far superior, less tedious, & less prone to error than drop counting.

This is a very interesting situation. Our graph has the form



Delay 2 min on pH reading.

A logistic curve works quite well.

$$pH = \frac{7.56}{1 + 1.448e^{(-0.1335 \cdot Vol)}}$$

$Mse = .017$

Volume is in grams.

Determining the inflection point here is of interest.

The system appears to act as a buffer beyond the inflection point.

Notice we have no sharp rise in pH here.

The inflection point does indeed occur essentially right @ the ~7.0 region (6.86 – 7.05, depending upon the point of selection).

The system therefore become an excellent jumping off point for study.

1. ~~Monoto~~ Monotonically increasing pH
2. No singular rapid equivalency point
3. Gradual convergence toward constant pH
4. Buffering capabilities demonstrated
5. An inflection point is present, interestingly enough at ~ pH 7.0.
6. The chemical reaction here is of interest.

This is a perfect place to begin the studies.
Acetic Acid + Sodium bicarbonate → ?
Stoichometry & pH analysis.

$$NaHCO_{3(s)} + CH_3COOH_{(l)} \rightarrow CO_{2(g)} + H_2O_{(l)} + Na^+_{(aq)} + CH_3COO^-_{(aq)}$$

The reaction actually has two steps to it.
Also CH_3COOH (preferred in my book) is also written as $HC_2H_3O_2$, which is not at all intuitive to me in terms of functional groups and structure.

CH_3COO^- is the acetate ion.

Remember the different definitions of an acid and base?
Hydrogen donors & electron acceptors
Look that up.

Guess what? The main buffer in the blood is

sodium bicarbonate!

There is no redox taking place here. There is dissociation. We have the acetate and the sodium ions being released. And we have gas & water being formed.

An acid serves as an H^+ donor.
A base acts as an H^+ acceptor.

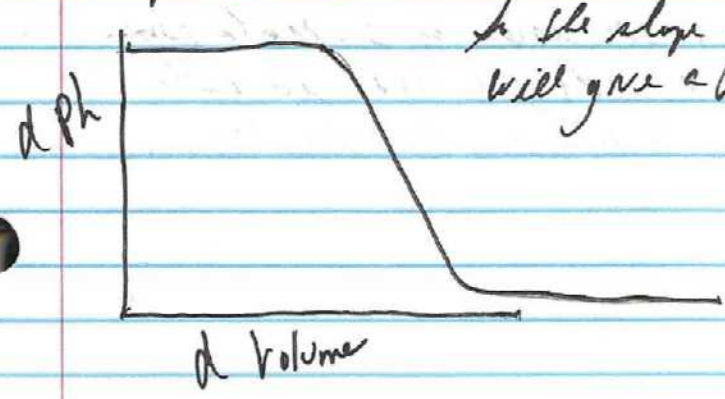
Notice acetic acid donates an H^+.
It is actually a rather strong acid (pH ~3)
Baking soda is a weak base. It can actually be either an acid or a base, but in water, it acts as a base:

$$NaHCO_3 + H_2O \rightleftharpoons \overline{H_2CO_3} + OH^- + Na^+$$

decompose to CO_2 & H_2O.

Interesting that no one seems to be looking @ a titration between the two in the way that I have,

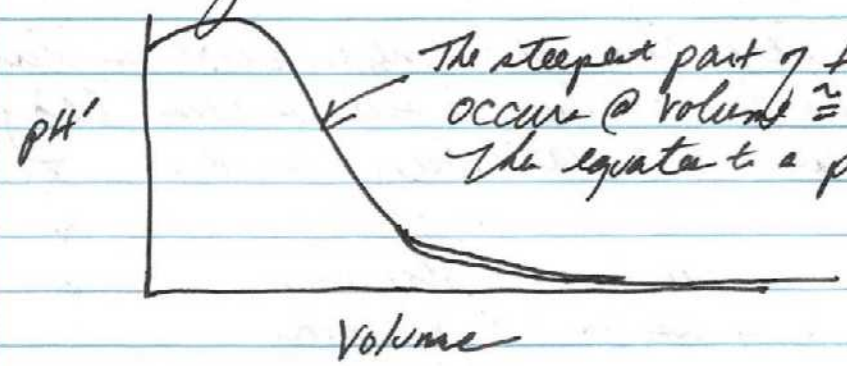
Slope Curve:



So the slope of the logistic curve will give a sharper

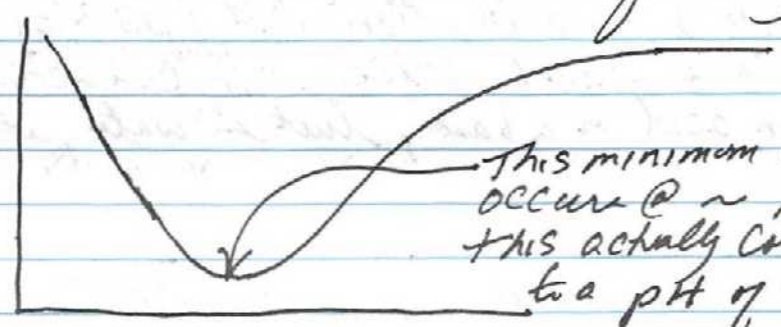
Jan
Dec 05 2019

With DPlot, I now have a differentiated curve available. The differential curve actually has the form:



The steepest part of this curve occurs @ volume ≅ 14.5
This equates to a pH of ~6.3.

The second derivative curve has the following form:



This minimum actually occurs @ ~ 12.5 volume
this actually corresponds to a pH of ~6.0.

Stoichiometry & pH & equilibrium points are worth it strongly.

This is therefore actually the best estimate for the equivalency point. This ends up to be a rather interesting reaction to find this minimum point as a second derivative is required to do so.

The next thing we notice is that this reaction produces a sodium ion and an acetate ion. This means that we should also be able to use conductivity to monitor this reaction. Let's try it.

Using 8 ml of vinegar in 40 ml of H_2O.
Using same baking soda solution prepared earlier

	Mass of Titrant (Baking Soda Solution)	Vol	EC (Conductivity) (2 min delay)	t (min)
*	19.22 gms ref		1.26	0
	17.56	1.66	2.78	2
	16.42	2.00	3.17	4
	15.27	3.95	3.22	6
	13.62	5.60	Ø.65 4 min delay	10
			Notice that the max has already been reached.	
	We see that time is		Ø.38 6 min delay - still stabilizing	12
	another variable in the		Ø.32 8 min delay	14
	reaction - 3 dimensional.		Ø.60 11 min delay. Nite increase now!	17!
	titrant			
to	EC = f(time, volume)		Ø.97 21 min delay	27 min stabilizing
	11.36	7.86	Ø.91	29 m
	7.85	11.37	1.01	31 m
*	18.82 ml	11.37+	—	33m
	13.00	17.19	.86	33m
	7.86	22.33	1.61	40m
*	18.45 ml	22.33+	—	—
	7.85	32.93	2.65	50m
*	18.79 ml	32.93+	—	—
	7.88	43.84	7.56	64 ~~60~~m [sharp rise]
		43.84	12.57	75m

Notice the strong increase @ the end of the run.

43.84 13.00 89 min

We see that, from a conductivity standpoint, this presumed simple & common reaction is actually quite complicated, electrically speaking.

We see great sensitivity @ the beginning of the reaction w/ a local maximum reached w/ a small volume of titrant.

Then we see a lagged decrease that eventually reaches a local minimum, w/ a gradual rise in conductivity after that point. We also see the importance of time that comes into the reaction @ this point.

From this point on we see a gradual increase in conductivity w/ a increased progression of concentration of the titrant, with a continued influence of time in the reaction.

This all demonstrates that a great deal of ionic equilibrating is taking place in the reaction from the moment it begins.

The reaction of sodium bicarbonate w/ acetic acid is, therefore, very much an kinetic reaction of equilibration and ionic exchange.

Therefore we see that pH & conductivity analyses can tell us a fair amount about the general nature of a reaction. Both acid base chemistry & ionic exchange are @ the forefront of the common reaction.

It is a question of whether the ORP probe reveals any activity.

I am also interested in the stoichometry and the pH analysis of the reaction.

—

Notice the sharp rise does not increase until the volume is quite high. The volume (i.e., ~45 ml) is way beyond the ~15 ml point tentatively identified as the acid-base equilibrium point.

This says the acid-base chemistry and the Na^+ – acetate ion formation are two completely different processes that are occurring concurrently.

The rapid rise to a local maximum very early in the reaction is very interesting also. We might presume that this is involving H^+, OH^- formation. This is substantiated by the rapid rise in pH @ the beginning of the titration also.

ORP Titration.
Same solutions.
8 ml vinegar in 40 ml of H_2O.
Same baking soda concentration.

	Time (m)	Vol (gms)	ORP mv	Vol
	0	0	+285	
*	0	19.07 ref	+285	0
	2	18.33	+280	0.74
	4	13.99	+250	5.08
	7	7.90	+190	11.17
*	—	18.84 ref	—	+11.17
	9	14.09	+160	15.92
	12	7.89	+110	22.12
*	—	18.95 ref	—	+22.12
	15	7.88	+130	33.19
*	—	18.47 ref	—	+33.19
	19	7.84	+150	43.92
*	—	18.66 ref	—	+43.92
	23	7.86	+150	54.72

This is a cubic relation.
Min occurs @ ~ 28.5 ml
$r^2 = 0.967$

Therefore, even though this is not known to be a redox reaction, we nevertheless do have a change in the ORP of the reaction. ORP, pH & EC all express different aspects of the reaction system. The local ~~and~~ min & max points vary for each attribute that is being measured.

The interpretation of titration attributes is, without doubt, a more ambiguous affair.

pH: You have seen that a sharp rise in the pH is not always going to occur. But you nevertheless have a significant inflection point that may indeed be the equivalency point of the reaction.

EC: Electrical conductivity. Quite a complicated affair however, with lots of variable activity. EC in this case is a function of both volume and time. The product generally will reflect information and exchange.

ORP: Also an interesting case in that the relationship develops in a cubic form. A local max is reached @ different volume levels for all attributes being measured. The interpretation of ORP is most uncertain @ the point, eq. since the reaction is not identified as a REDOX reaction.

All of this is entirely separate from electrochemistry investigations such as voltammetry, OCP, etc.

Now let us run a full trial w/ NH_3 & baking soda for a comparison.

Usual 8 ml of Vinegar in 40 ml Vinegar + total of water

Ammonia

$$\frac{20\text{ ml}}{50\ NH_3+H_2O} = \frac{60\ NH_3}{150\ (NH_3+H_2O)}$$

Void ~~The pH of the analyte (vinegar) is bouncing around quite a bit. Seems to be stabilizing @ ~5.4~~
OK, it is fine @ 2.7.

	t(m)	Vol (gms)	pH	Vol	t	Vol	pH	Vol
*	0	17.56 ref	2.67	0	16	15.87	8.53	41.69
	1	16.74	3.01	0.82	17	13.59	8.67	43.97
	2	15.73	3.26	1.83	18	7.85	8.89	53.42
	3	11.11	3.83	6.45	* 19	18.44 ref	—	+53.42
	4	7.85	4.06	9.71	20	7.85	9.13	64.01
*	—	17.66 ref	+9.71	—	+9.71			
	5	13.25	4.30	14.12				
	6	10.37	4.46	17.00				
	7	7.89	4.59	19.48				
*	—	18.51 ref	—	+19.48				
	8	12.62	4.94	25.37				
	9	7.86	5.35	30.13				
*	—	17.98 ref	—	+30.13				
	11	13.10	6.61	+35.01				
	12	10.82	7.97	37.29				
	13	9.50	8.22	38.61				
	14	7.85	8.41	40.26				
*	—	17.30 ref	—	+40.26				

We have a classic titration curve that appears here. None of the regressions are suitable, including logistic.

Our data point @ Vol = 35 is manually accepted as being the midpoint of the steep rise.

Vol = 35.01 pH = 6.61

We can, from here, determine the mass percent of NH_3 if assuming density of our analyte + titrant = 1 gm/cm3

Our vinegar is $\frac{8\ ml\ vinegar}{40\ ml\ H_2O} = 0.20$

Assuming Vinegar is 5% we have $0.20(5\%) = 1\%$ solution and we have the volume @ 40 ml

$(1)(40\ ml) = (X)(35.01) \Rightarrow X = 1.14\%$

but we have a dilution ratio of $\frac{60}{150} = 0.4$ and $\frac{1}{0.4} = 2.5$

So our estimated mass percentage of NH_3 is $(2.5)(1.14\%) = 2.85\%\ NH_3$

Household ammonia is between 5–10%
However another source says they are between 2–10%.
and my results are perfectly consistent w/ this.

In lieu of additional information, I will accept my determination as correct, i.e., $2.85\%\ NH_3$

You have an excellent titration curve

Now for Conductivity:
Vinegar: 8 mL in 40 mL total as the analyte
NH_3 same & carried forward as the titrant

	t	Vol	Vol	EC
uf	0	17.94 uf	0	1.16
		17.33	0.61	1.23
	1	15.96	1.98	1.54
	2	15.31	2.63	1.73
	3	13.78	4.16	2.13
	4	10.96	6.98	2.81
	5	7.84	10.10	3.45
*	—	18.25 uf	+10.10	—
	6	14.81	13.54	4.05
	7	7.85	20.50	4.95
*	—	18.26 uf	+20.50	—
	8	12.26	26.50	5.60
	9	7.82	30.94	5.97
	10	17.74 uf	+30.94	—
	10	7.85	40.83	6.81
*	—	17.94 uf	+40.83	—
	11	7.85	50.92	6.66
*	—	18.05 uf	+50.92	—
	12	7.86	61.11	6.11
*	—	18.49 uf	+61.11	—
	13	7.85	71.75	5.87

This is cubic. $r^2 = 0.997$
May occur @ 41 gms volume (mass)
Obviously 41 gms is a different point than 35 gms for pH.

Once again, we have a different process taking place. What is the reaction here?

$NH_3 + CH_3COOH \rightarrow NH_4^+ + CH_3COO^-$

NH_4^+ has accepted a hydrogen
CH_3COOH has donated a hydrogen

CH_3COO^- has lost a hydrogen

acids donate a hydrogen (make sense since they have H^+)
bases accept a hydrogen (OH^- is trying to make water).
Here, acetic acid donates a hydrogen, becomes CH_3COO^-
NH_4^+ has accepted a hydrogen, it is the base NH_3
has accepted a hydrogen.

The maximum ion concentration appears to occur with a volume of 41 gms titrant.

The maximum ion concentration is apparently not the same as the equivalency point in the reaction.

Next is ORP analysis. Prepare more ammonia.
60 ml NH_3
150 ml (H_2O + NH_3)

* Vinegar 8 ml Vinegar
40 ml (H_2O + Vinegar)

ORP Titration

	t(m)	Vol	ΣVol	ORP	
*	0	17.86 ml		+106	VOID
	3	1.01		+109	Notice, no effect whatsoever upon ORP.
*	4	18.17 ml		—	
	4	1.88		103	
*	—	17.77 ml		—	
	5	1.86		103	
*	—	18.55 ml		~~+103~~	To be repeated, error w/ cap
	6	1.85		103	
*	—	18.46 ml		—	
	7	1.84		105	

Summary:

	Acetic Acid + Sodium Bicarbonate Vinegar + Baking Soda	Acetic Acid + NH_3 Vinegar + Ammonia
pH	Monotonic increase, Inflection point Vol ≅ 13 gms 2nd derivative minimum	Classic titration curve Proposed equivalency point @ pH=6.61 Vol=35.0 gms
EC	Complex pattern of local max & min Strong ionic increase @ end of run. No single equivalency point.	Cubic pattern Max local @ Vol=47 gms
ORP	Cubic relationship. Local min @ 28.5 gms Vol	~~No~~ Void No effect of any kind observed! Linear relationship r^2=.993

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Dec 06 2019

The demonstrates how different each reaction is. The relationship at very widely for each reaction and each process used.

Obviously pH, EC & ORP titration can be used to establish a "signature" for a reaction. Interpreting the behavior is a different matter.

Repeat ORP Run:

	t(m)	Vol	Vol	ORP	
*	0	18.32 ml	0	+313	There is a linear relationship. $r^2 = .993$
	1	15.45	2.87	310	
	2	11.97	6.35	304	
	3	7.88	10.44	295	
*	—	17.58 ml	+10.44	—	
	4	12.46	15.56	285	
	5	7.88	20.14	276	
*	—	18.34 ml	+20.14	—	
	6	7.90	30.58	252	
*	—	17.98 ml	+30.58	—	
	7	7.85	40.71	236	
*	—	18.56 ml	+40.71	—	
	8	7.85	51.42	197	
*	—	18.46 ml	+51.42	—	
	9	7.85	62.03	165	
*	—	18.88 ml	+62.03	—	
	10	7.90	73.01	144	
*	—	18.80 ml	+73.01	—	
	12	7.83	83.98	111	
	18		83.98	83	

Notice that both reactions investigated are completely different in behavior w.r.t. pH, conductivity and ORP.

There are several interesting points to make here.

1. Remember that the dissociation of NaCl itself is a redox reaction - recall Tyler video, so the topic needs to be entirely reinvented as ORP logging shows significant activity even in ionic reactions.

2. What you are actually dealing with here is also the branch of Chemistry called reaction KINETICS. You see that every reaction here is very much a function of time. It may not be so obvious w/ certain acid-base reactions, but as you see you should never assume that time is irrelevant.

Reaction kinetics are included w/in one lab I look & have @ the lab - I do not have that experiment w/ me but it involves the oxidation of blue food color. I might be able to recreate the experiment but the discussion would be valuable here. Concentrations will end up being important in the process as the "order" of the reaction is assessed.

3. Our regressions are giving us insight into what that titled "role" is. Logistic regressions are a separate matter.

We definitely need to look into the topic of reaction kinetics, from what we already see.

4. There must definitely be a "kinetic signature" of a reaction that can be described here using the attributes of pH, EC & ORP. Furthermore, the specific concentrations of analyte vs titrant further uniquely define the reaction with the specific regressions that can be developed from the measurements.

5. Quite a lot of effort could be extended here to interpret the kinetics of these reactions, and I am sure it could be quite insightful & write the principles of

pH 1. Acid - base chemistry
EC 2. Ion formation & exchange (re electron transfer)
ORP 3. Oxidation - reduction reactions

These three methods comprise an absolute mainstay of chemical reaction study.

6. In addition to the kinetics of these reactions, there is much to be gained by basic chemistry study here of

1. Lewis structure
2. Formal charge
3. Molecular geometry (a focal target)
4. Functional groups

Help determine function, in chemistry, as in proteins, as in life in general.

7. As a total addendum, look into esters and proteins - the curiosity comes from the sleep state.

Looking @ Chemical kinetics as an overview, it indeed is an interesting and can be quite an advanced topic. The conventional approach is to measure reactant concentrations while I am measuring indirectly via, pH, EC or ORP.

I do find a paper from ACS called:

"Reaction kinetics from conductivity data: An apparatus for the student laboratory".

so, indeed, it is possible...

I can access 50 papers w/ my account. If I can get logged in, fantastic...

We do see that "conductometric methods for chemical kinetic studies have been well documented in the past" ... from the retrieved ACS paper.

I also see some references for the use of ORP w/ kinetics

Vernier has some very good experiments w/ reaction rates and equivalent point using conductivity
The general Vernier lab book and our Advanced Chem lab book by Vernier both have good experiments in them.

—

Ok, I have found some very good resource to pursue various titrations. There are a series of experiments that have been published and they do go beyond acid-base chemistry.

—

For now, I would like to back up and look at Lewis structure, formal charge & molecular geometry. Also lets back up on the redox question w/ ionic reactions.

—

Our first reaction is:

$$NaHCO_3 + CH_3COOH \rightarrow CO_2 + H_2O + Na^+ + CH_3COO^-$$

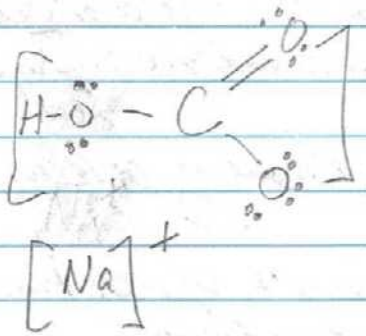
Lewis Structure
$NaHCO_3$?



There is indeed somewhat unusual & different.

It is resonant.

$NaHCO_3$



Lewis structures apparently do not work well w/ ionic substances.

A resonant structure

Lets look @ formal charge:

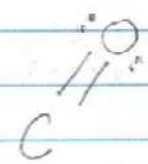
In this case, you have the formal charge so you can construct the Lewis structure in reverse.

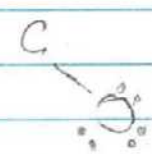


$[HCO_3]^1$

Formal Charge = ~~Wants~~ Should have (Valence Electrons) − Has Directly Attached Electrons to Atom

	Valence		Attached	
H:	Wants	−	Has	= ∅
	1		1	
O	Wants	−	Has	= ∅
	6		6	
C	Wants	−	Has	= ∅
	4		4	
O_2	Wants	−	Has	= 0
	6	−	6	
O_3	Wants	−	Has	
	6		7	= −1





Work w/ formal charge further:
The problem w/ Leah's method is that it already presume that you have the Lewis Structure in Place.

Ok, I am seeing it. The Lewis structure and the formal charge go hand in hand. The method to help construct the Lewis structure is

Formal Charge = Should Have Valence Electrons − Directly attached electrons

H⇒	1	−	1	=0
O⇒	6	−	6	=0
C⇒	4	−	4	=0
O_1⇒	6	−	6	=0
O_2⇒	6	−	7	=-1

Here is how you go both ways:

1. If you have the formal charge, you construct the Lewis structure to make it accordy to
Formal charge = should have valence electron (will always know this) − has directly attached electron

2. If you have the Lewis structure, you can determine ~~Construct~~ the formal charge.

Scenario no 1. will be more common.

also:
Directly attached electron = Should have valence electron − formal charge
6 − (-1) =7

Now it is really interesting to me that no one actually shows a Lewis structure for the complete molecule, even though it is a solid molecule and not dissociated in that form. They are always keeping the Na^+ as a separate entity. Lewis structures do not seem to be applied to ionic compounds very well?

Now, let's go to the geometry:
Steric number: I II III ...

2 sp
3 sp^2
4 sp^3

Structure around the Carbon: No lone pairs
Steric no = 4 $\Rightarrow$ sp^3 hybridization
I think tetrahedral? ~~This is correct.~~
NO!

Oxygen: Steric no = 4. Lone pairs = 2 (Symmetric?
No, it is bent, both electrons
point to the opposite side.

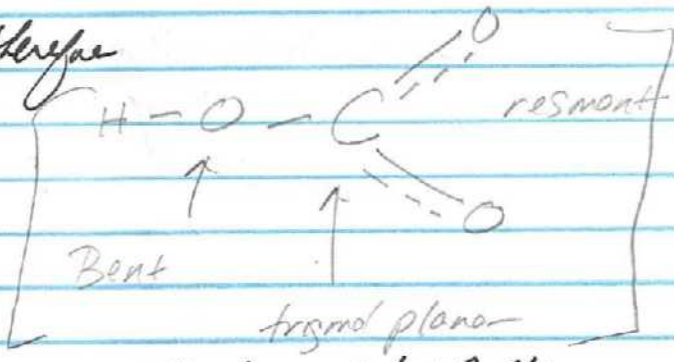
It is true that the O atom has a bent geometry. But because of the resonance of the oxygen bonds the geometry is trigonal planar (ie apparently sp^2 w/ no lone bond pairs).

This is the bi - Carbonate ion.

I have a molecular modeling program which provides an answer.

-1

The actual structure is therefore



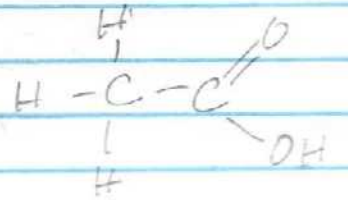
And then supposedly the Na^+ ion is left hanging out by itself, both disocciated +1

[Na]

Dipole moment = 2.46
also highly electronegative.

this is now reacting w/ acetic acid (dilute in H_2O)

CH_3COOH



The oxygen region is much more electronegative
Dipole moment = 3.02

Formal Charge:

C_1: Should Valence − Has Direct = 4 − 4 = ∅
C_2: 4 − 4 = ∅
O: 2 − 2 = ∅
Σ = ∅

Geometry: Stearic No: / // /// ..

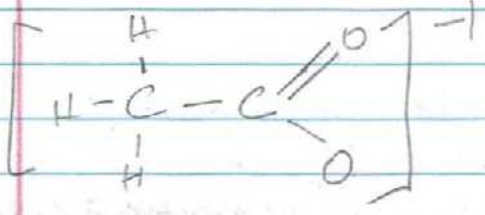
C_1 :	4	⇒ sp^3	no lone pairs	tetrahedral	✓
C_2 :	3	⇒ sp_2	no lone pairs	trigonal planar	✓
OH :	2	⇒ sp	two lone pairs	bent	✓

The reaction is:

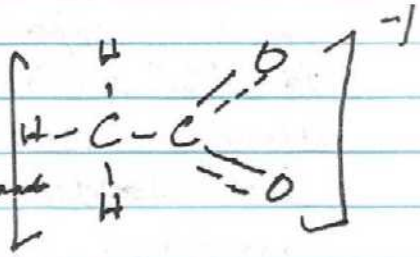
$$NaHCO_3 + CH_3COOH \rightarrow CO_2 + H_2O + Na^+ + CH_3COO^-$$

The acetate ion becomes a resonant structure w/ trigonal planar geometry around the second carbon.
The dipole moment increases to 4.04 (highly electronegative)

Let's look @ formal charge and Lewis structures and geometry of $[CH_3COO]^{-1}$



Actually it is resonance



Formal Charge:

C_1:	$4-4=0$	Geometry: I II III ..			pairs / me
C_2:	$4-4=0$	✓ tetra C_1: S.N. = 4	sp^3		0
O_1:	$6-6=0$	✓ trig planar C_2: SN = 3	sp^2		0
O_2	$6-7=-1$				
	$\Sigma = -1$				

You had an OH previously, so you had geometry there as bent. You no longer have OH.

Ok, I am starting to get a handle on this. An important question is to develop an application and understanding as to how the geometry becomes important.

Here are two simple examples.

1. CO_2 is linear & therefore non-polar. Therefore it does not dissolve in water well.

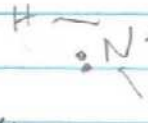
2. H_2O is a bent molecule and is therefore polar: The effects cohesion, surface tension & hydrogen bonding.

Proteins, DNA, enzymes have many important factors related to their shape and geometry.

Lets look @ what we learned this year.
Baking soda was only moderately polar compared to acetic acid. Notice how soluble acetic acid was compared to baking soda. Notice also the dipole moment of the acetate ion.

Reaction rate also depends upon the shape.
A change in structure changes the dipole moment.

Look @ NH_3



Formal charge: Should (Valence) − Has (Direct)
5 − 5 = 0

Geometry: Steric = I II III ..
Steric No = 4, 1 lone pair
trigonal pyramidal – yes. It also is polar.
Dipole moment = 1.49

The seems fairly moderate. The would indicate only a modest solubility of NH_3 in H_2O.

Ok, that's better. Now we can proceed to some titration experiments + discussions.

I see that temperature is another way to perform a titration. We have

1. Conductivity - Conductometric
2. pH
3. ORP
4. Temperature
5. OCP - Potentiometric.

Dec 07 2019 Review Titration Experiments & Methods

1. Temperature is another way to approach titration. A good thermometer (resolution 0.01°) is required. We have one. Paras - Advanced Chemistry Sensor: pH, US, temperature, pressure

2. Titration experiment: H_2SO_4 + $Ba(OH)_2$
   I have these available also.

3. Titration experiment: H_2SO_4 + $Ba(OH)_2$
   In addition to US measurement, the precipitate mass is collected, dried & weighed as an alternate method of concentration determination.

4. One next titration example is that of a conductometric titration between a strong acid and a strong base. Notice that a pH meter is NOT used and that conductivity titration is completely viable for acid-base titrations. There is important new information w/in the titration method description.

   The principle of conductometric titration is that during the titration, one of the ions is replaced by the other and that this causes a variation in conductivity of the solution.

   We are after maximizing the acuteness of the angle between the two branches of the titration curve.

More specifically:

1. The smaller the conductivity of the ion which replaces the reacting ion, the more accurate will be the result. Thus, it is preferable to titrate a silver salt w/ lithium chloride rather than HCl. Generally, cations should be treated w/ lithium salts and anions with acetates as these ions have low conductivity.

2. The larger the conductivity of the anion of the reagent which reacts w/ the cation to be determined, or vice versa, the more acute is the angle of titration curve.

3. The titration of a slightly ionized salt does not give good results, since the conductivity increases continuously from the commencement. Hence, the salt present in the cell should be virtually completely dissociated. For a similar reason, the added reagent should also be a strong electrolyte.

4. Non-linear titration curves result. This is due to a continuously increasing volume.

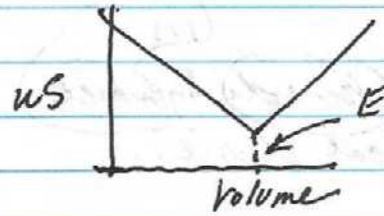
5. The change in volume should be kept small, and titrant should be 10-20 times stronger than the analyte.

6. Advantages of Conductometric titration is that it can be used on very dilute and colored solutions, and also to systems that involve incomplete reactions. Potentiometric titrations cannot do this, such as the neutralization titration of phenol ($K_a \cong 1E-10$). Conductometric methods can be used here.

7. Acid-base titrations at trace levels can be carried out w/ conductometric methods. Error $\leq 1\%$.

8. There are few disadvantages w/ this technique.

During conductometric titration of a strong acid-strong base:
1. Initial conductance is high due to mobile H^+
2. When base added, conductance falls due to replacement of H^+ with the formation of undissociated H_2O. The decrease continues until the equivalence point.
3. At equivalence point, solution only contains NaCl (NaOH + HCl).
4. After equivalence, conductance increases due to the high conductivity of OH^- ions.



This was a helpful discussion.

Now, even though we were using a pH meter on the initial titration of ~~Dec 04 2010~~ Jan 04 2019
* Leading to a Monotonically Increasing Titration Curve, this could well be related to the previous discussion.

In our case we were titrating vinegar w/ baking soda.

Vinegar: Ka = 1.8E-5
Baking Soda: Ka = (carbonic acid)

Carbonic Acid: Ka = 4.37E-7 (the fine titrant was weak of the two)
Sodium Carbonate: Ka = 4.68 E-11

Sodium bicarbonate & sodium carbonate mixed in water make a buffer solution.

The anion is a negatively charged ion
The anion is attracted to the anode.
Remember "An Ox" & "Red Cat"
Oxidation takes place @ the anode.
Reduction takes place @ the cathode.

Cathode is the negatively charged electrode by which electrons enter in an electrical device.

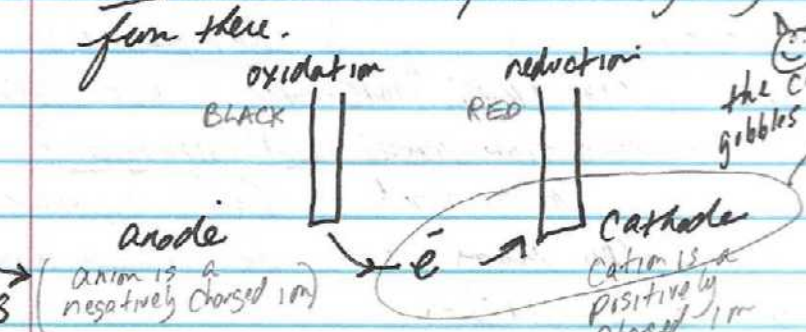
No wonder this is confusing. The difference between actual current and "conventional current" is totally prone to confusion. My interest is primarily in actual current.

So an essence to understand is that

electrons enter into a cathode.
Let's see if this makes sense w/ "An Ox" & "Red Cat"

If oxidation is the loss of electrons, then that causes the movement of those electrons toward the cathode, where they enter it. That is the process of reduction, which means that it is gaining the electrons. The loss of the electrons make sense, occurring @ the anode, where oxidation is taking place. It all makes sense with actual current.

All that is actually required to remember is that electrons ENTER the cathode, and everything can be constructed from there.



is all you need to remember.

also makes sense → Now in Conventional current, it flows from "positive to negative"

BUT CONVENTIONAL CURRENT IS OPPOSITE! to actual current. This means the electrons are actually entering the red terminal, and therefore the red terminal on a battery is actually accepting the electrons and is therefore the cathode!

No wonder this is confusing. Just remember the cat gobbles up electrons and you can reconstruct everything.

You have it right

Now, and only now, we can go back to the general principle of conductometric titration, and understand what is being said.

"The larger the conductivity of the anion of the reagent which reacts w/ the cation to be determined (or visa versa), the more acute the angle of the titration curve".

Now, let's look @ this in more detail.

~~Incidentally, it seems to me that any time that you have movement of electrons, you are going to have oxidation and reduction of some form taking place. This seems like it almost always will be happening to some degree.~~ NOT TRUE. "MOVEMENT" IS NOT SUFFICIENT. OXIDATION STATE MUST CHANGE!

Negative! VOID!

Before we go further, let's look @ Tyler again with the redox video using NaCl as an example.

$$Na^{0} + Cl^{0} \rightarrow \overset{+1\ -1}{NaCl}$$

These two atoms are electrically neutral!

Na gives an electron to Cl

leads to

Na^+ Cl^-

these stick together

Na^+ Cl^-

The reaction is crucial. The formation (or dissociation) of salt is indeed a redox reaction!!

Na is being oxidized

Cl is being reduced.

Now, this gets interesting as I am interested in the reverse process. The issue lies in the application of external energy. External energy will allow the redox process to take place; without it it will not. The reverse process of dissociation is not a redox process, the oxidation state does not change during dissolution. You can also understand the ionic salt reaction.

$NaCl \rightarrow Na^+ + Cl^-$

$Na^\circ + Cl^\circ \rightarrow Na^{+1}Cl^{-1}$

$Na^\circ \rightarrow Na^{+1} + e^-$ $+2.711\,V$

$Cl^\circ + e^- \rightarrow Cl^{-1}$

or $Cl_2 + 2e^- \rightarrow 2Cl^{-1}$ $+1.359\,V$

highly! $\mathcal{E} = +4.070\,V \geq 0$ ✓

Clearly the reaction does ~~not at all~~ proceed spontaneously. The reaction in reverse ~~also~~ does not proceed, only dissolution will occur. The net voltage would be highly negative. Interestingly though by electrolysis of sufficient voltage, it should proceed. In that case, we would therefore, have a redox process take place. The clarifier matter. Dissociation is not redox, a change in oxidation state must take place. Recall this take place @ discrete energy levels.

Therefore, MOVEMENT of electrons IS NOT sufficient to signify a redox reaction taking place.

A change in oxidation state MUST take place to consummate a redox reaction.

At this point we can go back to the quoted titration statement.

We know that the anion is a negatively charged ion.
We know that it leaves the anode.
We know that it migrates toward the cathode.
We know that the cathode accepts electrons.

Now, looking back @ our vinegar, we had

$H_2O + CH_3COOH \rightarrow H^+ + CH_3COO^- + H_2O$

In our baking soda we have

$H_2O + NaHCO_3 \rightarrow Na^+ + HCO_3^- + H_2O$

We looked @ Lewis structures, formal charge & molecular geometry of these molecules.

We know the net reaction is
$NaHCO_3 + CH_3COOH \rightarrow CO_2 + H_2O + Na^+ + CH_3COO^-$

So the question is, what exactly is happening @ the equivalence point?

For the case of a strong acid and a strong base, @ the equivalency point $H^+ = OH^-$ concentration is equal, and these combine to form water, so at the equivalency point you only have salt & water.

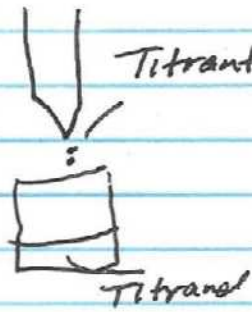
and pH = 7.0

The situation w/ a strong acid/weak base, this well not be the case.

Strong base/weak acid] these shift the equivalence point to
Strong acid/weak base] a pH which is not 7.0.

He uses titrant (being added) and titrand (added to)
At equivalence point (titrand) (titrant) (we use analyte also)

@ Equivalence Point (1) pH < 7 weak base by a strong acid
However @ the equivalence point, the moles of strong acid equal the moles of a weak base.
(titrand) (titrant)



@ Equivalence Point (2) pH > 7 weak acid by a strong base
At the equivalency point, the moles of the strong base = the moles of the weak acid.

In case (1) Henderson Hasselbloch equation can only be used before the ~~equilibrium~~ equivalency point is reached.

Interesting Notes

At the equivalency point, you need to use an equilibrium expression to determine the pH.

The half equivalence point is where the pH = pKa. Looks to be an important point in the titration process.

Flipping titrant & titrand also causes a mirror image of the titration curve, and pH relationship reverses also.

Now we understand what the equivalency point means, we

moles of acid = moles of base

whether a not we or the other is strong or weak.
We should also be able to predict what type of titration we performed by an analysis of the Equivalency point.

Let's take our first case of vinegar + baking soda
B: Vinegar was the titrand.
Baking soda was the titrant.
We ended up with an equivalency point of pH 7.85
this implies that we started w/ a weak acid
and added a stronger base.

In fact, our vinegar was rather diluted and the
baking soda solution was fairly concentrated.
This would be the rationale @ the time.

—

Notice however, our derivative analysis
led us to a different conclusion. Here we
have a minimum occurring, via a second
derivative analysis, @ the pH of 6.0, and thus
is stated to be our best estimate of the equivalency point.

This says that our titration was that of a weak
base by a strong acid.

There is actually much more logical sense we know that vinegar has a pH of ~ 3.5 and baking soda has a pH of ~ 8.

So guess who is stronger? Relatively speaking, vinegar is indeed a strong acid & baking soda is a weak base.

This is the more logical conclusion.
We have the measurement to determine concentration.

$\frac{8\text{ml } 5\%\text{ vinegar}}{40\text{ ml of H}_2\text{O + vinegar}} = (0.20) \Rightarrow (0.20)5\% = 1\%$ vinegar.

$M_1 \cdot V_1 = M_2 \cdot V_2 \Rightarrow 40\text{ml}(1\%) = M_2(12.5\text{ml (measured)})$
$M_2 = 3.2\%$ Baking Soda
Let's see what we weighed out. We determined it to be 5.86%
So this is not atrocious, but I would also hope that it would be closer than this.
But notice that the pH of the baking soda titrand is 7.85%, exactly the same as the inflection point on the titration curve. This would hardly be the equivalency point as well. Not sure of the reason for the large difference.

Notice that our conductometric titration gave a very reasonable result w/ $V_2 = 7.86$ ml for a minimum reached.
$M_2 = \frac{(1\%)(40\text{ml})}{7.86\text{ml}} = 5.09\%$ - fairly close.

It appears that the conductometric titration was successful and the pH titration only marginally so. A monotonically increasing pH was hardly a good theory so this make sense to some degree. It also assumes a 5% vinegar solution.

The ORP also looks like a marginally successful titration as it presented a monotonically decreasing titration curve.

Of the three titrations, the conductometric titration is the only one that established a definite minimum in a logical & consistent fashion.

Notice also that during the discussion of the conductometric titration that indeed a minimum is expected to be attained; an acute angle indicates a more accurate titration.

Notice that we also had a second min on the EC titration (@0.86 in addition to @0.91) which introduces some uncertainty into the titration. It might be beneficial to reproduce the titration.

The titrant should be ~ 10 times the strength of the titrand; we are @ ~ 5 times so we might be able to improve the results by weakening the acid further. We could use ~~4 mL~~ in 8 mL of vinegar in a total of ~ 100 mL of H_2O; this should represent an improvement.

Repeat the titration under these modified conditions.

Now we look @ the NH_3 - Vinegar titration.

Firstly, by the pH titration method, we did establish a classic titration curve format. We also have an equivalency point determined @ ~ 35 gms w/ a pH of ~ 6.61.

The samples we are working w/ a weak base and a strong acid, and these match the scenario. A concentration level of ~ 3% is determined for NH_3 and these are also not unreasonable.

The EC titration also reaches a local maximum (note not a minimum in this case) @ ~ 41 gms. This would lead to a slightly weaker NH_3 concentration determination, but is also not out of bounds.

We then notice that the ORP titration is only linear and monotonically increasing. No apparent value.

The different methods of titration (ORP questionable, however) all appear to be useful in one form or another in helping to determine an equivalency point.

It appears that it may be helpful to weaken the acidic solution and repeat the pH & EC titrations.

The next question that arises is what if you have a mixture in the analyte (titrand)? It is possible that multiple equivalency points may be reached here.

It does seem possible that molecular weight can be determined by titration also. Is that the case?

The answer is yes. There is an unexpected and amazing benefit of the process.

Example: 0.4955 gms of a monoprotic acid are neutralized by 37.06 ml of 0.100 M NaOH?

so $\frac{0.4955 \text{ gms acid}}{? \text{ mol acid}} = ? \text{ gms/mol}$

So how many mole of acid (again again assuming monoprotic)

$$H(?)_{(aq)} + Na(OH)_{(aq)} \rightarrow H_2O + Na(?)_{aq}$$

The ? is some unknown ion. After all, an acid is just a proton, an H^+, and an anion.

37mol 37 ml of 0.1 m NaOH = ?

$$37 \text{ ml NaOH} \left(\frac{0.1 \text{ mol NaOH}}{1000 \text{ ml NaOH}}\right) \cdot \frac{1 \text{ mol H(?)}}{1 \text{ mol NaOH}} = .0037 \text{ m H(?)}$$

and molecular mass = $\frac{0.4955 \text{ gms}}{.0037 \text{ mol}}$ = $\frac{133.9 \text{ gms}}{\text{mol}}$

"Titrations are really stoichometry problems, but the formula of the acid is unknown".

This is a very powerful method. Very practical.

However, notice that the no. of gms of the acid are known here, not the no. of grams within a solution

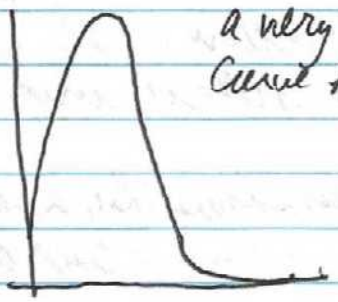
Let's repeat the titrations:
Now 8 ml in 100 ml H_2O on vinegar.
Baking soda: Use ~ 9 gms in ~150 ml
Mass of Container 127.22 gms
135.26 w/ baking soda
Δ = 8.04 gms
280.62 w/ H_2O = 153.40

$H_2O + NaHCO_3 \Rightarrow \frac{8.04}{153.40} = 5.24\%$

	t	Vol 17.39	Vol	EC
*	0	~~17.3 ref~~	17.41 = 0	1.12
	2	16.56	0.85	1.28
	4	15.57	1.84	1.66
	8	14.68	2.73	1.98
	10	13.58	3.83	2.31
	18	12.09	5.32	~~2.36~~ 2.31
	21	10.90	6.51	2.25
	25	10.26	7.15	1.99
	29	9.34	8.07	0.32
	33	8.62	8.79	0.00
	36	7.88	9.53	0.00
*	—	18.01 ref	+9.53	—
		16.84	+10.70	0

	t	vol	Vol	EC
	38	7.88	19.66	0.00
*	—	19.02 ref	+19.66	—
	40	7.83	30.85	0.00

We have a maximum @ 4.6 gms.



a very peaked curve this time

Our vinegar solution is $\frac{8\text{ ml}}{100\text{ ml}}(5\%) = 8\%$

$= .08(5\%) = 0.40\%$

$100\text{ ml}(.40\%) = 4.6\text{ ml}(x) \quad x = 8.7\%$
vs measured 5.2% Still too much difference

Ok, our equivalence point is not @ 4.6 ml
It is the sharp drop off @ ~ 7.5 ml

$\frac{100(0.4\%)}{7.5\text{ ml}} = 5.3\%$ vs measured 5.2%

That is it. The sharp drop off is the equivalency point. Good work.

So what is the local maximum @ ~ 4.6 ml?

The run demonstrates the high level of success w/ the conductometric titration under ideal conditions. Notice also that our modification of a weaker vinegar solution was highly successful, as from the notes on the conductometric method mentioned earlier.

It also makes the point that the molecular structure of the unknown (sodium bicarbonate) was not required to determine the mass percentage.

It also shows that a highly dilute titrand (analyte) solution can be used as also mentioned.

The means that there is no need to determine the no of moles present to obtain required data. Molar knowledge requires structural knowledge.

Next let's repeat the NH_3 titration.
Formerly we used 60 ml NH_3 / 150 ml (H_2O + NH_3)
In the past run, we had a cubic relationship.
8 ml vinegar / 100 ml (H_2O + vinegar)
NH_3 60 ml NH_3 / (H_2O + NH_3) @ 150 ml

	t	vol	VOL	EC	
*	0	18.25 ml	+H2O	1.14	Cubic solution $r^2 = .997$
	4	17.11	1.14	1.20	Local max occurs @ 42 ml 9 ms.
	7	15.85	2.40	1.39	
	13	13.62	4.63	1.75	$M_1V_1 = M_2V_2$
	17	10.39	7.86	2.27	$100(.42) = M_2(42ml)$
	19	7.86	10.39	2.65	$M_2 = 0.95\%$
*	—	17.84 ml	+10.39	—	
	21	13.07	15.16	3.34	The does not seem very
	25	7.85	20.38	4.01	likely. In addition, it
	—	18.33 ml	+20.38	—	is a local max instead
	29	13.22	25.49	4.60	of a local min or
	31	7.86	30.65	4.92	a sharp drop off.
*	—	18.49 ml	+30.65	—	
	44	12.95	36.39	5.17	It is unknown what
	46	7.86	41.02	5.11	the significance of the
	—	18.18 ml	+41.02	—	local max shown is.
	51	4.88	54.32	4.87	

Not sure what to repeat here or why.

Jan
Dec 08 2019

NH_3 - Acetic acid via EC (Conductivity) provided no defendo result. We reach a shallow maximum and no local minimum.

We do get results (not useful) that are similar to the previous EC run on NH_3 - ~~H_3COOH~~ $HNCO_3$
Notice that our last results came through w/a pH meter, therefore it seems beneficial to try multiple methods. Repeat the pH titration.
Vinegar 8 ml / 100 ml (H_2O + Vinegar)
NH_3 60 ml / 150 ml ($NH_3 + H_2O$)

	t	vol	VOL	pH	t	vol	VOL	pH
	0	0	0	3.14	–	17.48 ml	+40.95	–
*	–	17.71 ml	0	–	21	7.82	50.61	9.09
	3	16.30	1.410	3.41				
	5	14.85	2.86	3.70	Steepest part of			
	6	13.09	4.62	3.90	curve is @ 33 gms.			
	9	10.87	6.84	4.08				
	10	7.88	9.83	4.27	$100(.4\%) = (33ml)\ X$			
*	–	18.71 ml	+9.83	–				
	13	13.57	14.97	4.55	$X = 1.21\%$			
	14	11.19	17.35	4.10				
	15	7.85	20.69	4.86	Seems rather low but			
	–	17.01 ml	+20.69	–	we do have a reasonable			
	16	13.52	25.04	5.10	titration curve here.			
	17	7.88	30.68	5.60	We would like the NH_3 to			
	–	18.14 ml	+30.68	–	be stronger and/or the			
	18	12.52	36.30	7.68	CH_3COOH to be weaker.			
	19	10.57	38.25	8.28				
	20	7.87	40.95	8.62				

Realize however, we used here 100 ml of solution.
~33 ml (gms) ⇒ ~ 3 to 1 ratio instead of ~ 10-20.

A couple of suggestions. If you use full strength NH_3 your ratio will be closer 1 to 1. Improved but still not best.

Suggest we:
Put in $\frac{1}{2}$ ml of vinegar in 50 ml H_2O] Done
Use full strength ammonia.

	t	vol	VOL	pH
*	0	18.48 ml	0	3.28
	1	18.22	0.26	3.69
	4	17.54	0.94	4.36
	6	15.81	2.67	5.52
	8	15.00	3.48	8.36
	15	14.62	3.86	8.78
	18	12.87	5.61	9.39

$\frac{2}{50} = \frac{1}{25} = .04$

$.04(5\%) = 0.2\%$ Vinegar

50 ml

$M_1V_1 = M_2V_2$

$(.2\%)(50) = M_2 \cdot (\sim 3) \approx 3.3\%$

OK

Equiv Point ≈ 3.1 by inspection.

Therefore $50\text{ ml}(.2\%) = M_2(3.1\text{ gms}(\sim\text{ml}))$

$= 3.2\%$

Our first determination was 2.85%. $\bar{X} = 3.02\%$

Therefore our best estimate of the NH_3 concentration is ~ 3.0% which seems quite reasonable.

We now have two titrations involving two different reactions that appear to have produced fairly reliable results.

One titration result handled confirmed by an alternate direct measurement of concentration.

The second titration has confirmation w/ a repeat measurement under low error conditions.

We learn that multiple methods, trials & deletions are likely required to bring a successful titration to the forefront.

—

Now that we have an initial introduction under our belt, also preceded with

Lewis structure
Formal Charge
Molecular geometry

} to accompany it.

We can now consider venturing into molecular weight determination by titration. This is an intriguing prospect.

Lets look into the molecular weight question a bit?
Anyway we know the mass of acid that is used. (Not the case by a long shot). So we assume that it is monoprotic.
What is monoprotic? - Contains only one hydrogen in its formula.

Back to example. Assume 37 ml of 0.1m NaOH was used to neutralize. How many moles is this?

$0.1m = 0.1\left(\frac{40gms}{mol}\right) = 0.4\frac{gms}{liter} = \frac{X}{37E\text{-}3\ l}$ $\quad X = .015E\text{-}3\ gms$

$\frac{.015E\text{-}3\ gms}{liter} = \frac{.015E\text{-}3}{40}$ $\quad \frac{40gms}{liter}$ = 1m NaOH $= \frac{40gms}{1000ml} = \frac{X}{37ml}$

$\frac{40gms}{liter}$ $\quad X = 1.48gms$ for 1M NaOH. But we have 0.1M

We have $\frac{0.148gms\ NaOH}{37ml}$ how many moles is this? $\frac{.148gms}{40gms/mol} = .0037m$ OK

We have $\frac{.0037\ moles}{4o\ in\ 148gms} \cdot \frac{1\ mole}{x}$ $\quad x =$

The neutralized ~~one mole of the acid~~ .4955 gms of the acid

$\frac{.4955gms\ acid}{.0037\ moles} = \frac{X}{1\ mole}$ $\quad X = \frac{133.92gms}{mole}$

neutralized

The is the molecular weight of the hypothetical acid.

Now, our problem is the acid is dissolved in water.
What if we tried to subtract the water?

Our activation is actually more akin to

$$\left(\frac{X \text{ gms acid}}{X \text{ grams acid} + 100\text{ml } H_2O}\right) = \frac{y}{1 \text{ mole}}$$

$$\frac{}{.0037 \text{ moles}}$$

$$y = \left(\frac{X \text{ gms acid}}{X \text{ grams acid} + 100\text{ml } H_2O}\right) = \frac{\text{gms}}{\text{mole}}$$

$$\frac{}{.0037 \text{ moles}}$$

if you assume X gms acid is small relative to vol of H_2O you have

$$y = \frac{X \text{ gms acid}}{X \text{ gms} + 100 \text{ gm } H_2O} = \frac{\text{gms}}{\text{mole}}$$

$$\frac{}{.0037 \text{ moles}}$$

if we solve for mass percent, then we do have the quantity.

That can be done it seems.

Lets try the idea, at least to some approximation.

Sodium Carbonate

$HNaCO_3 \rightarrow Na^+ + H^+$

Let's make either NaOH or KOH .1M solution:
We will use KOH. MW = 56.106 gms/mol

$1M = \frac{56.106\ gms}{liter} = \frac{x}{200\ ml}$ $x = 11.221\ gms$

I do not want to use that much. Let's make 100 ml of .05M KOH. $1M = \frac{5.611\ gms}{100\ ml}$ $.05M = \frac{0.281\ gms}{100\ ml}$

$.1M = \frac{0.561\ gms}{100\ ml} = \frac{1.12\ gms}{200\ ml\ total\ (KOH+H_2O)}$ use this.

Weigh boat = 2.72 gms
+ 1.12 gms
3.84 gms

Empty jar mass: 187.94 gms
+ 200
387.94 gms

At. 200 ml of KOH 1M solution has been made ($KOH+H_2O$)
Our best available acid to neutralize a most likely vinegar.
Let's run a trial run of 20 ml vinegar.

t	vol	VOL	pH
* 0	15.24 ml		2.46
1	14.77		2.50
2	14.09		2.61
3	12.41		2.84
	10.61		

The vinegar is too strong.
Dilute by a factor of 5.

Now we have 4 ml vinegar in 20 ml (H_2O + vinegar).
and 0.1 M KOH

t	vol	VOL	pH
0	18.32 ref		2.79
1	17.54		3.04
2	16.14		3.36
3	14.71		3.56
3	12.98		3.74
4	10.04		3.96
4	7.85		4.08
* —	18.54 ref		—
5	13.24		4.33
6	7.83		4.55
—	18.03 ref		—
	7.89		4.92
* —	18.60 ref		—
9	7.84		5.46
—	18.60		—
11	15.38		5.75
12	13.58		5.98
12	11.98		6.29
13	11.08		6.56
14	10.48		6.82
15	9.60		7.59
16	9.19		8.95
17	7.88		10.01

Equivalency point @ ~ 63 ml

This is still way too weak of an acid.

Recommend
4 ml of vinegar in 20 ml (H_2O + Vin)
1M Solution of KOH

100 ml should be sufficient

= 5.61 gms / 100 ml (KOH + H_2O)

2.72 weigh boat
+ 5.61 KOH
8.33 gms

$\frac{5.61 \text{ gms KOH}}{100 \text{ ml } (H_2O + KOH)}$ = 1M KOH

Again

t	vol	VOL	pH
0	18.19		2.90
1	17.84		3.62
2	17.47		3.98
3	17.03		4.21
3	16.71		4.37
4	16.06		4.65
4	15.45		4.91
4	14.91		5.14
5	14.40		5.50
6	14.22		5.65
6	14.05		5.87
7	13.87		6.29
7	13.61		10.31

Equivalence Point $\cong$ 4.45 ml

% KOH = 5.61%

So

~~20 ml (5.61%) = (X%)(4.45 ml)~~

Vinegar is

$\sim 5\% \left(\frac{4}{20}\right) = 1\%$

so $(20\text{ ml})(1\%) = X\%\,(4.45\text{ ml})$

$X = 4.49\%$ KOH

We calculate it to be 5.6%

measure

vs Calculated 4.5%

Error = 1.1% Acceptable.

Our moles of KOH:

We have a 1M solution = $\frac{56.11 \text{ gms}}{1000 \text{ ml}}$ $\times$ 4.45 ml $\quad x = 0.250 \frac{\text{gms}}{\text{mole}}$

$\frac{0.250 \text{ gms}}{56.11 \text{ gms/mol}} = .0044$ moles KOH

VOID

$\frac{4.49\%}{.0044}$ $\frac{.0449 \text{ (\% conc.)}}{.0044 \text{ moles}} \stackrel{??}{\cong} 10.2 \Rightarrow$ Multiply by dilution ratio = 20/4 = 5

$5(10.2) = 51 \frac{\text{gms}}{\text{mol}}$

MW of $CH_3COOH = 60.05 \frac{\text{gms}}{\text{mol}}$

Maybe?

Remember the vinegar is a 1% solution? Remember it was diluted by a factor of 20/4 = 5

??

and

$\left(\frac{20}{4}\right) \frac{.0561}{.0044} = 63.7 \frac{\text{gms}}{\text{mol}}$

We are entitled to use the 5.61% mass concentration since that was weighed out on a scale. If we consider the dilution ratio of the acid then we are surprisingly close

$$\left(\frac{20}{4}\right)\frac{.056\,l}{.0044\ \text{moles}}\left(\text{KOH Concentrate}\right) = \frac{63.7\ \text{gms}}{\text{mol}}$$

vs actual $60.05\ \frac{\text{gms}}{\text{mol}}$ → Is this justified?

Jan
DEC 10 2019

Molar Mass Determination by Titration - Study further
if you know the ratio

Molar Mass = $\frac{\text{grams}}{\text{moles}}$ moles of acid = moles of base
require 1 to 1 ratio
of HCl + NaOH

certain oriented toward a solid acid.
You always need to determine the no. of mole to neutralize.

$\frac{\text{grams}}{\text{moles}} = \frac{x}{1 \text{ mole}}$ x = molecular mass.

NaOH can be applied to a weak acid.
So in the case, it is straight forward when the
mass of the acid is known. But we never know the.
The primary relationship is mole acid = mole base.
We need relationship between mole & mass %.

$\text{No. of mole} = \frac{\text{grams of sample}}{\text{Molar mass}}$ $\text{Decimal mass \% by weight} = \frac{\text{No. of grams}}{\text{Mass of (solvent + Sample)}}$

so

Grams of sample = No. of mole * Molar Mass

Grams of sample = $\text{Mass \% by weight}_{\text{Decimal}}$ * Mass of (solvent + sample)

No. of mole * Molar Mass = $\text{Mass \% by weight}_{\text{Decimal}}$ * Mass (solvent + Sample)

or

Molar Mass = $\frac{\text{Mass \% by weight}_{\text{Decimal}} * \text{Mass (solvent + sample)}}{\text{No of Moles}}$

These are all determinate. The should be possible.

Let's try to apply this using a conventional approach.
Given

24.68 ml of Ø.1M NaOH neutralizes Ø.2931 gms of an unknown acid.

First, how many moles in ~~26~~ 24.68 ml Ø.1M NaOH
Molar Mass of NaOH = 40gms

$$Ø.1M \text{ solution} = \frac{(Ø.1)(40gms)}{1000ml} = \frac{4gms}{1000ml} = \frac{X}{\cancel{26}\ 24.68\ ml}$$

But we only have 26 ml.
so X = ~~Ø.104 moles~~ .099gms and $\frac{.099gms}{40gms} = .002475$

you forgot this step properly.

Now assume we dissolve the acid in 100 ml of H_2O.

$$\frac{Ø.293gms}{100gms(H_2O + acid)} = .00293 \text{ mass \% (decimal) by wkg}$$

We should therefore be able to solve the molecular mass as:

$$\text{Molecular mass} = \frac{(.00293)(100gms)}{\cancel{.104 \text{ moles}}\ \cancel{.002408}\ .002475} = \cancel{2.817}\ \cancel{121.7}\ 118.4 \text{ gms}$$

(gms of sample / Molecular Mass)

Actual answer is:

This is superb. The method will work.
The example answer is: 118.7 gms

You have it!

Your method is superb and it works very well.

So you have a modified method that is able to use a mass % by weight in combination w/ the no. of moles required to neutralize the acid.

(Notice this is the mass % by weight)

Molar Mass = Decimal Mass % by weight * Mass (Solvent + Sample) / No of moles required to neutralize a 1:1 ratio.

Everything here is known. This is great. Lets start to apply this.

Lets look @ our previous attempt

We measure that 4.45 ml of 5.61% (Mass Wgt) KOH neutralizes the vinegar (1% solution)

We neutralized w/ KOH

1M KOH = 56.11 gms / 1000 ml but we have a ~~.0561 (dec)~~ 1 Molar solution

$= \frac{56.11 \text{ gms}}{1000 \text{ ml}} = \frac{x}{4.45 \text{ ml}}$ $x = 0.25$ gms

and remember this step: Convert to mole NaOH: $\frac{.25 \text{ gms}}{56.11 \text{ gms}} = .00445$ mole

We can now solve for molecular mass.

Molecular Mass = $\frac{(.0561 (\text{dec}))}{.00445 \text{ (moles of base)}} * 100 = 1261$ gms / mole

if it was pure acetic acid but it is not!

Vinegar

$CH_3COOH = 60.05$ gms / mole

But remember, we have a 5% solution!

$.05(1261) = 63$ gms / mole

I think we have it!

It apparently will be possible to find the molar mass of a diprotic acid from titration (your choice).

You would now like to repeat your method w/ a different acid.

Which one would you like to use?
How about citric acid? Boric Acid?

Citric Acid $C_6H_8O_7$ $C_6H_8O_7$
Boric Acid H_3BO_3 (weak inorganic acid)
Ascorbic Acid $C_6H_8O_6$ = 176.12 gms/mol

Now, in this case, we will be able to weigh both boric acid & ascorbic acid samples. We should have two ways of solving.

Weigh boat: 2.72 gms
w/ascorbic acid 7.63 gms
4.91 gms ascorbic acid

We have therefore made a 4.91% mass by wgt solution of ascorbic acid. We will use 20 mL of this solution as the titrand.

Our pH of the solution is ~ 2.49.

We shall neutralize w/ 1M KOH

Titration of Ascorbic Acid w/ KOH (pH meter)

t	vol	VOL	pH
1	18.37	0	2.45
2	18.10	0.27	2.81
3	17.82	.55	3.06
4	17.47	.90	3.29
5	17.05	1.32	3.49
6	15.71	2.66	3.94
7	13.93	4.44	4.40
8	11.82	6.55	5.12
9	10.42	7.95	10.02
10	10.88	7.49	10.88
11	7.88	10.49	11.50

Equiv. Pt ~ 11.12 = 7.25 ml

A 1M solution of ascorbic acid is 176.12 gms/mol. We are assuming a 1 to 1 ratio in the chemical reaction.

$= \frac{\cancel{176.12}\ 56.11\ \text{gms}}{1000\ \text{ml}} = \frac{x}{7.25\ \text{ml}}$

$x = \cancel{1.277}\ .407\ \text{gms} = .00725\ \text{moles}$

Our mass % of the solution is 4.91%. Therefore:

NO

$$\text{Molecular mass} = \frac{(.0491)(100\ \text{ml})}{.00725\ \text{moles}} = 677.2\ \frac{\text{gms}}{\text{mol}}$$

Dec mass ← NO needs to be 27 ml

If we had a 4 to 1 ratio, this is the only way this will work

Now, realizing our equivalence is occuring @ ~27 ml with the added volume, we have

This makes sense of final actual volume

$$\frac{(.0491)(27\ \text{ml})}{.00725\ \text{mole}} = \text{Molar Mass} = 182.8\ \text{gms/mol}$$

actual

OK

vs actual 176.1 gms/mol

And this works.
This suggests we use the final actual volume of titrand, which actually makes sense.
We have a 4% error which is reasonable.

It appears that a viable method is developing
This is a very powerful stoichiometry - titration method.

Jan
Dec 11 2019

Now let's try citric acid.

Weigh boat: 2.72 gms
~~4.77 gms w/ citric acid~~
4.74 gms w/ citric acid
Δ = 2.02 gms Citric acid. = 4.04 gms / 100 ml
in 50 ml (H_2O + Citric acid)

I use only 20 ml of the solution
Titrate w/ 1M KOH

	t	Vol	VOL	pH	
*	0	18.27 ml	0	1.98	Neutralization @
	1	18.02	.25	2.13	Total Volume of
	2	17.49		2.41	33.19 gmol (~ml)
	3	15.90		3.03	(Weighed)
	5	13.77		3.80	Since we used
	6	12.36		4.25	20ml our equivalency
	8	10.91		4.69	point is therefore
	9	9.36		5.19	33.19ml − 20ml
	10	7.84	10.43	5.66	= 13.19 ml
*	11	17.50 ref	+10.43	—	
	12	16.60		6.00	If we assume the 1:1
	13	16.31		6.12	molar ratio we have:
	13	15.86		6.36	↑ Not the
	14	15.50		6.63	case.
	15	15.20		7.10	
	16	14.93	13.00	9.41 ←	
	17	14.52		11.47	

KOH 56.11

1M NaOH = 40gms / 1000ml = x / 13.19ml x = 0.528gms / 0.740gms

0.740

No. moles = 0.528 / 56.11 gms/mol = 0.0132 moles Neutralize the Citric acid.

Molar Mass = (decimal .0404) 23.19ml (total volume) / .0132 moles = 101.58

(Assuming 1:1 mole ratio!) ——— Not the case!

Citric Acid is $C_6H_8O_7$ = 192.1 gms/mol

So what happened here? We could have a molarity of 2 to 1?
What is the reaction here?

1 mole citric acid + 3 mole KOH → One mole of potassium citrate + 3 mole of water.

$C_6H_8O_7 + 3KOH \rightarrow C_6H_5K_3O_7 + 3H_2O$

So it is definitely not a 1:1 mole ratio
But it is 3 to 1, not 2 to 1. So what did happen here?

—

The next question is how to handle a non 1:1 molar ratio.

—

We learn that the method only works for monoprotic acids & monobases. (1 proton and 1 OH in the formulas).

Here we go:

Titrations (Any ratio)

$$\frac{M_{acid} \cdot V_{acid}}{n_{acid}} = \frac{m_{base} \cdot V_{base}}{n_{base}}$$

n is the no. of moles of acid and base in the balanced chemical reaction.

$$\frac{M_{acid} \cdot V_{acid}}{1} = \frac{m_{base} \cdot V_{base}}{3}$$ in our case

so $M_{acid} \cdot V_{acid}(3) = m_{base} \cdot V_{base}$

$$m_{base} = \frac{M_{acid} \cdot V_{acid}(3)}{\cancel{V_{base}}\ V_{base}}$$

This says to me that our equation should be of form?

$$\text{Molar Mass} = \frac{\left(\begin{array}{c}\text{Decimal Concentration} \\ \text{of Titrand}\end{array}\right)\left(\begin{array}{c}\text{Total Final Volume of Titrand}\end{array}\right)}{\text{moles titrant} \cdot 3}$$

which leads to a

Molecular mass of 33.86??? off by a factor of $\frac{192.1}{33.86}$

$= 5.7???$

I do not know where this is coming from ongoing to Let's find the polyprotic video discussion.

There is an excellent case to see how the pH software analyzes this case.

First off, it appears as though citric acid is a triprotic acid.
3 pKa's, 3 pKb's & 3 protonation constants.
Incidentally, ascorbic acid has two protons, diprotic.
Concentration in software are required in molarity.
We have a citric acid concentration (mass %)

$$\frac{2.02\,gms}{50\,ml\,(H_2O + CitAcid)} = \frac{4.04\,gms}{100\,ml\,(H_2O + CitAcid)} = 4.04\%$$

$$\frac{2.02\,gms}{50\,ml} = \frac{x}{1000\,ml} \Rightarrow x = 40.4\,gms \Rightarrow \frac{40.40\,gms}{192.1\,gms/mol} = .21\,M$$

Our final volume @ equiv. point is 33.19 ml.

From base, we have a 1M solution.
We have used 13.19 ml to reach equivalency point.

Software solution says that at this point we have
4.2E-3 ~~.00244~~ moles of citric acid ratio ~~= 12 to 1~~ 5 to 1
.0132 ~~.03319~~ moles of KOH where as it should be
~~5 to 1, to~~ 3 to 1??

There we go. Now we have it.

The ratio of $\frac{.0132 \text{ moles of KOH}}{4.2E-3 \text{ moles of citric acid}}$ is 3.14 to 1
Now we have it.

Our titration curve from the software shows a pH @ the equivalency point as being

9.50 vs our measured 9.41
so we have done quite well.

We have most certainly properly identified the equivalency point.

Now we assume the titrate we have
MW = $\frac{\text{grams}}{\text{mole}}$ = $\frac{0.8069 \text{ grams (software calc)}}{.0132 \text{ mole (software calc \& our calc)}}$

Thus leads to a MW of $61.1 \frac{\text{gms}}{\text{mole}}$

and 61.1 (3) = 183.3 gms/mole vs 192.1 actual.

But we did not notice the 61.1 — we got 33.9 gms/mole

Let's back up. We know we agree w/ the .0132 moles of KOH required to reach the equivalence point.

Now let's look @ the .8069 gms. of the acid.
Let us calculate how many grams
We know we have a $\frac{4.04 \text{ gms}}{100 \text{ ml}}$ solution concentration
but we used only 20 ml.
$\frac{20}{100}$ (4.04 gms) = .808 gms.

Note: Our stoichiometry software has balanced this reaction w/ ease.

There is also a match. So we have some type of problem w/ the numerator of our equation.

Molar Mass = (Decimal Concentration) · Volume of Titrand Commencing!
mole of neutralizing base

$$= \frac{\frac{4.04\text{ gms}}{100\text{ ml}}(20\text{ ml})}{.0132\text{ mole KOH}} = \frac{.808\text{ gms acid}}{.0132\text{ mole KOH}}$$

= 61.2 gms per mole (but we have 3 moles of KOH per 1 mole of Citric acid Produced

$= 61.2(3) = 183.6\frac{\text{gms}}{\text{mole}}$ vs theoretical $191.2\frac{\text{gms}}{\text{mole}}$

Great! We have it. $\frac{183.6-191.2}{191.2} = 4\%$ error

Also suitable & reasonable error.

We have now successfully determined the molecular weight of a triprotic acid.

All cases are now successful.

You will not know yet if the protein acid is monoprotic, diprotic, triprotic, etc.

* We do NOT use the total volume of titrand, we use the beginning volume.

Jan
Dec 12 2019

I am making good progress now w/ the determination of the molecular weight of a broad variety of acids, including weak acids & poly-protic acids. The include
1. acetic acid
2. ascorbic acid
3. citric acid

w/ general high levels of success.

Lets now try boric acid. You are not, with the protein, going to have a known chemical reaction or even a known mass (we will improve this w/ the evaporation of water) but I shall mark w/ what I look. If you know the chemical reaction known, work out the stoichometry for an aid in the verification of results.

Boric Acid: H_3BO_3 looks to be tri-protic
Molecular mass: 61.83 g/mol

$H_3BO_3 + KOH \rightarrow H_2O +$

H_3BO_3 is not a "traditional acid".

$H_3BO_3 + KOH \leftrightarrow K^+ + B(OH)^{4-}$??

Then is a journey from a traditional reaction from all that I can gather.

We can run experiment w/ strong acids, but organic acids are a very interesting topic in themselves.

Let's work w/ HCl. Supposedly I have 10M HCl reasonably calibrated. Let's use 1 ml in 50 ml H_2O.

50 ml flask: 52.10 gms.
Add 1 gm HCl (~1 ml)
Brought mass to 50 gms.
For some reason it reads closer to 51 ml but will double and go w/ now. Assume we are using a 2% mass solution of HCl.
Titrate w/ KOH. I will expect high error.

t	vol	vol	pH	
9	18.39		1.04	so it has a very sharp rise.
10	18.14		1.00	Repeat.
12	10.68		1.50	
12	7.85		1.00	

Equivalent Point (pH = 7.0)
~ 9.2 ml
$\Rightarrow$ vol = 10.08 ml

50 ml flask: 52.66
1.00 ml ~10M HCl + vol to 50 ml (H_2O + HCl)

t	vol	vol	pH	t	vol	vol	pH	
~~1~~	~~19.28~~		~~1.06~~	8	11.20		1.64	21, 8.78, 10.72
2	19.28		1.03	9	10.68		1.81	
3	18.87		1.03	11	9.88		2.39	
3	17.85		1.05	12	9.63		3.21	
3	16.58		1.08	13	9.55		4.81	
4	14.98		1.15	14	9.44		5.71	
4	13.78		1.23	15	9.31		6.41	
5	13.05		1.31	16	9.27		6.71	
6	12.51		1.37	16	9.15		8.36	
7	11.68		1.51	18	9.10		9.23	
				19	9.04		9.61	
				20	8.88		10.28	

KOH

1M NaOH = $\frac{56.11 \text{ gms}}{1000 \text{ ml}} = \frac{X}{10.08 \text{ ml}}$ $X = 0.566$ gms KOH

$\frac{.566 \text{ gms KOH}}{56.11 \text{ gms}} = .010$ moles used.

Molecular mass = $\frac{(\text{Decimal Conc}) \cdot \text{Volume Titrand}}{\text{Moles to Neutralize}}$ $\frac{\text{Mass}}{\text{Original}}$

=

We used a 10M solution of HCl w/1 ml diluted to 50 ml.

HCl molecular mass = 35.45 + 1.01 = 36.46 gms/mol

so we have $\frac{(10)(36.46) \text{ gms}}{1000 \text{ ml}} = \frac{X}{100}$ $X = 36.46$ % solution by mass

and $\frac{36.46 \text{ gms}}{100 \text{ ml}} = \frac{X}{1 \text{ ml}}$ $X = 0.365$ gms in one ml

~~so our molecular mass is~~ $\left(\frac{\text{Dec Concentration}}{.3646}\right)$.

but we added to 50 ml so we have

$MW = \frac{\left(\frac{.365 \text{ gms}}{50 \text{ ml}}\right)(50 \text{ ml})}{.010 \text{ moles KOH}} = \frac{36.5 \text{ gms}}{\text{mole}}$

The actual molecular mass is $\frac{36.46 \text{ gms}}{\text{mol}} = \frac{36.5 \text{ gms}}{\text{mol}}$

Thus is superb work on this one.

We have now determined, with quite reasonable success, the molecular weights of 4 different acids of varying nature. The determination of a strong acid, HCl, was especially accurate.

When we go to the protein, we will not know the mass, the water content, or the chemical reaction. This will present some major challenges but we will push it forward as much as possible. Water can eventually be evaporated.

I also determined the molecular mass of HCl by the freezing point method, so the two different methods have now been used for a strong acid.

I think that we are ready to move onto the protein now. I will most certainly need to prepare additional 1M KOH.

$$= \frac{56.11 \text{ gms}}{1000 \text{ ml}} \quad = \frac{5.61 \text{ gms}}{100 \text{ ml}}.$$

residual

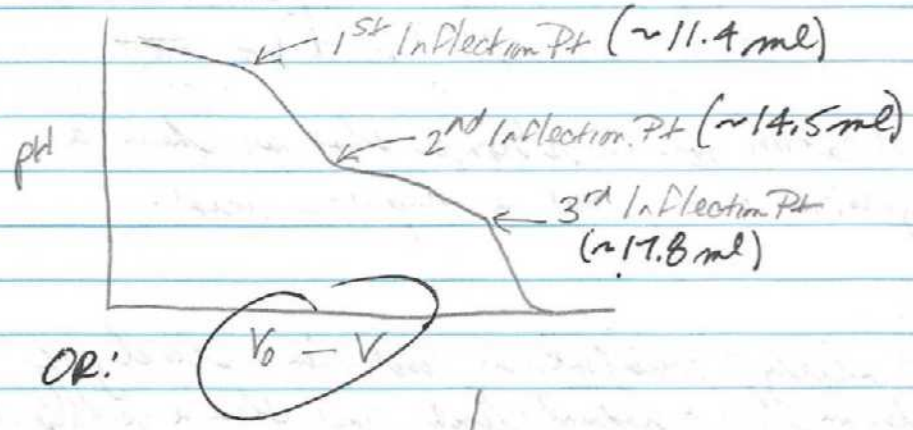
Our current solution mass + container is 231.11 gms
Weigh boat used to mass out 5.61 gms. - Successful.
236.74 gms w/ KOH added.
Want 331.11 gms total. Done. I have it.
I have now extended the 1M KOH solution. Stoichometry knowledge & a tare scale are very useful.

Ok start now to work w/ the protein.
Mass of 150 ml beaker: 127.15 gms.
Mass of beaker + protein. 130.69 gms Δ = 3.54 gms
Now bring to 20 ml of H_2O = 127.15 + 20 gms = 147.15 gms
Done! Titrate w/ 1M KOH

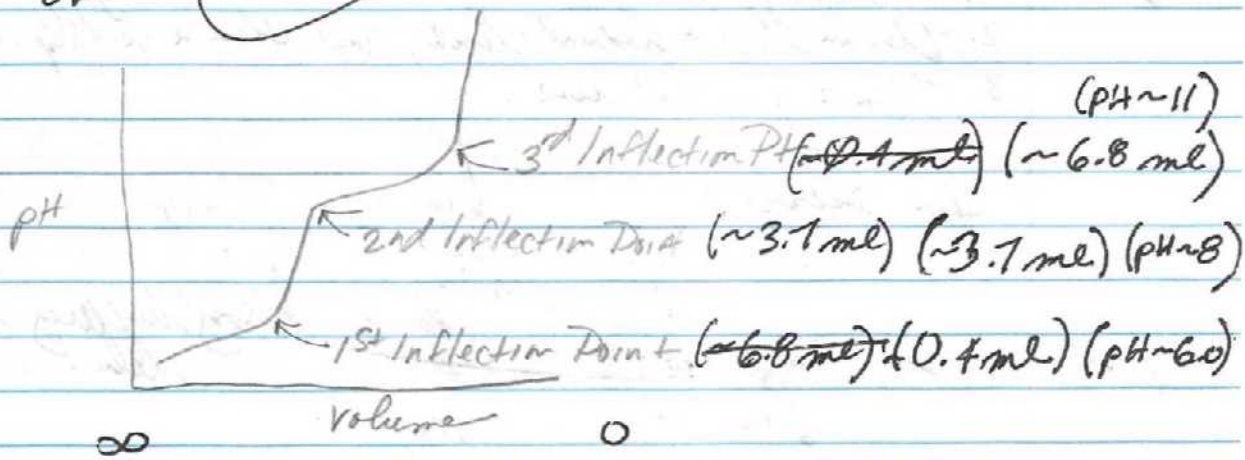
	t	vol	VOL	pH	
*	0	18.20 ml	0	2.52	tan - brown color
	2	17.97	Ø.23	3.60	
	4	17.92	Ø.28	4.38	Possible precipitate formation
	5	17.81	Ø.39	6.37	Solution change color
	8	↓	Ø.39	~6.00	
*	17	↓	Ø.45		
		↓	Ø.39	5.66	
	19	↓	Ø.39	5.57	
	22	17.73	Ø.47	6.83	
	25	↓	Ø.47	6.37	Acting like a buffer.
	28	17.66	Ø.54	6.96	Dark green now
	31	↓	Ø.54	6.56	
	33	17.60	.60	6.83	
	35	17.49	.71	7.04	
	37	17.36	.84	7.07	
	38	17.28	.92	7.05	Notice stability at now
	39	↓	.92	7.11	peak
	40	17.18	1.02	7.09	
	41	↓	1.02	7.25	peak
	43	17.08	1.12	7.31	peak Dark green
	44	16.93	1.27	7.32	peak
	49	16.84	1.36	7.40	peak
	50	16.76	1.44	7.49	peak
	51	16.67	1.53	7.55	peak
	54	16.49	1.71	7.64	stable

	t	Vol	VOL	pH	
	~~16.5~~ 56	16.34	1.86	7.69	stable
	59	16.09	2.11	7.81	
	62	15.55	2.65	~~8.02~~	stable
*	63	14.81	3.33	8.03	
	69	13.86	~3.6 mL 4.34	8.67	
*	71	12.87	5.33	10.18	We have a rise here.
	73	11.95	6.25 ~~~11.4 mL~~	~~10.88~~	
*	74	10.50	7.70	11.33	
	75	9.39	8.81	11.57	
	76	7.85	11.35	11.83	Very dark green. No settled precipitates.

There is a very interesting titration Complex behavior.



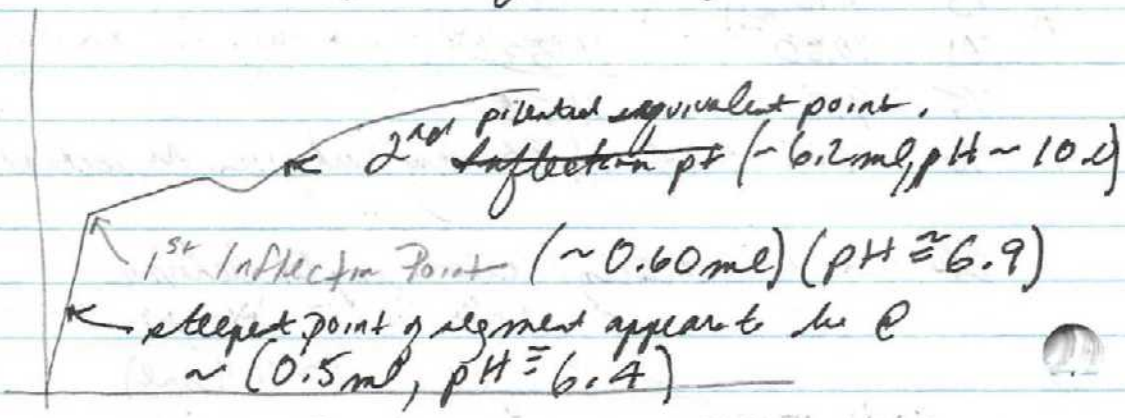
OR:



Jan 13 2019

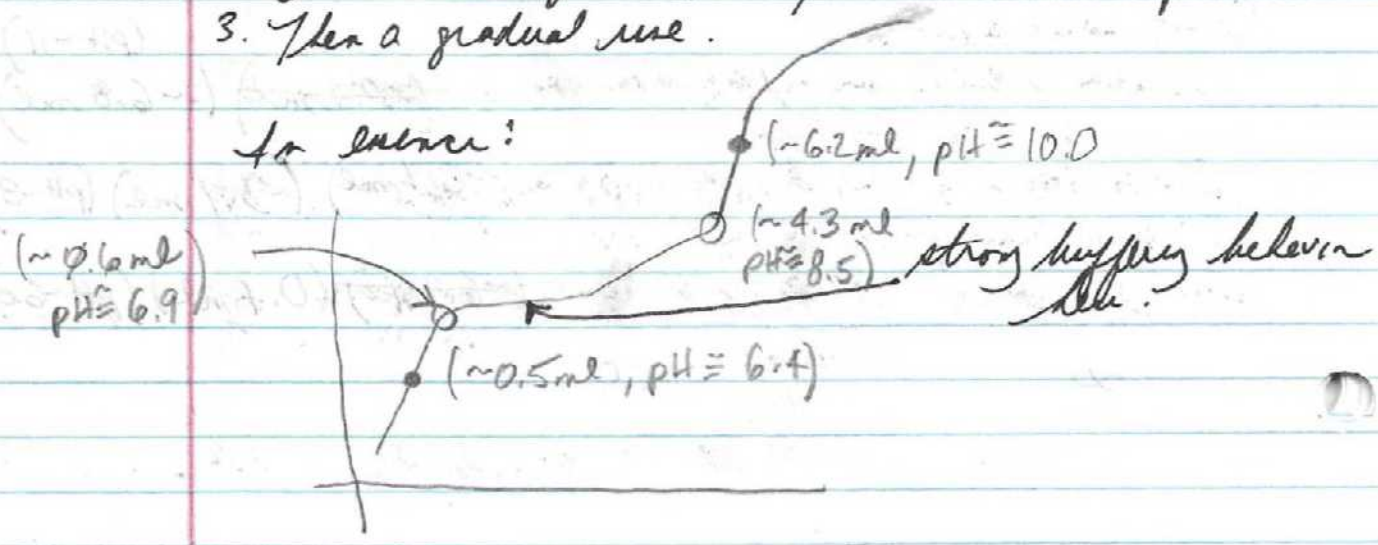
Let's report our data from yesterday - this is an important data set. It'll take a lot of work to produce sufficient quotient.

Now we know the volume formed correctly.
Our titration curve is of the following form.

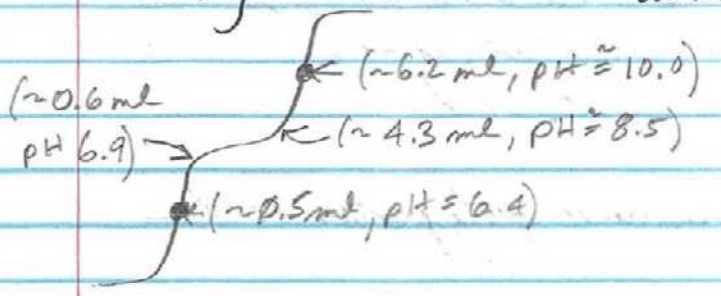


The indications now are stronger that we have a diprotic acid, not a triprotic acid.

We have
1. A steady & consistent rise and then a barely
2. Then we have a gradual climb, and then a steady rise
3. Then a gradual rise.



So now we think we have a diprotic acid. H_2A
Hardly a ideal titration curve of



However, we have matched it to the form as best as we can.
Now we learn to interpret the best that we can and ask how to approach molecular weight.

Let's determine the no. moles KOH @ each point to neutralize.

$$1M\ KOH = \frac{56.11\,gms}{1000\,ml} = \frac{X_1}{0.5\,ml} = \frac{X_2}{6.2\,ml}$$

$X_1 = .028$ gms Moles: $\frac{.028\,gms}{56.11\,gms/mol} = .000499$ mole KOH

$X_2 = .348$ gms Moles: $\frac{.348\,gms}{56.11\,gms/mol} = .00620$ mole KOH

Mass Concentration
unknown until complete evaporation takes place.
Assume for now 95% H_2O.
~~(.05)/(20 gms (ml) = 1.00~~ $.05(3.54\,gms) = .177\,gms$
This was dissolved in 20 ml of H_2O

Assume 95% H_2O!

$MW_1 = (\text{estimate})\ \frac{.177\,gms}{20\,ml} \cdot 20\,ml / .000499\,m = 355\,gms/mol$

$MW_2 = \text{estimate}\ \frac{.177\,gms}{20\,ml} \cdot 20\,ml / .00620\,mole = 28.5\,gms/mol$

This says the low volume neutralization corresponds to a higher molecular weight acid and the higher volume neutralization corresponds to a lower weight acid.

Let's watch discussions on polyprotic acids.

We learn that the final titration volume is what is required to compute the no. of moles for neutralization.

The general reaction is

$$H_2A + 2KOH \rightarrow K_2A + H_2O$$

A is the acid ~~cation~~ anion (a ~~cathode~~ anion has a ~~positive~~ negative charge since it is the source of electrons)
Electrons enter the cathode is all that you need to recall.

This means 2 moles of KOH are used for every mole of HA. Therefore when you compute the no. of moles of KOH to neutralize, the no of moles of HA will be one half of this.

This will cause the MW to increase by a factor of 2. We saw this before w/ a triprotic acid but did not really understand the direction of this factor.

So far now, we are assuming the protein is 95% H_2O.
We shall work on the problem.

But based upon this, our earlier Computation was
MW = 28.5 gms mole.
But now we understand it would be $\frac{\left(\frac{.111 \text{ gms}}{.00620 \text{ gms/mole}}\right)}{2}$
$\cong$ 2 57 gms mole.

If the water content is 90% vs 95% then
the MW estimate will increase by a factor of 2.
I will determine this.

searching
Then we start analyzing the molecular weights of
various diprotic acids to seek a match.

We are on the right track here.

Protein Mass Determination:
Weigh Boat 5.00 gms
Weight w/ Protein added: 11.85 gms
Δ = 6.85 gms before evaporation. 2045
Evaporation is underway. Started Dec Jan 13 2019 2030.

While the is proceeding over the next several days, I should proceed with an a Conductivity titration.

Again, use approx 3.5 gms in 20 ml H_2O for the protein.

3.66 gms protein
20.00 gms (protein + H_2O)

The first thing we learn is that the protein is highly conductive: $EC_0 \cong 10.68$

	t	vol	vol ml	EC	
*	0	18.99 g		10.73	
	1	18.83		10.40	
	2	18.66	0.33	10.26	minimum achieved
	4	18.41		10.40	Turns dark green
	6	18.26		10.50	
	7	17.96		10.67	
	8	17.42		11.04	
	9	17.06		11.29	
	~~10~~ 11	16.63		~~11.4~~ 11.72	
	~~13~~ 15	16.00		12.32	
	19	15.19		12.81	
	22	14.48		12.89	
	~~26~~ 27	13.34	5.65	15.30	sharp rise
	29	12.88		15.83	
	~~32~~ 33	11.32	7.67	18.45	sharp rise again
		9.95		Exceed meter - still a sharp climb	

We need to reduce the titrand concentration by ½ & ⅔
Dilute the KOH from 1M to ~~0.5M~~ 0.2M.

We have points of interest here @
~0.33 ml 0.5 ml
~5.65 ml vs 6.2 ml
~7.67 ml
EC Titration pH Titration

So now we drop protein to ~1.0 gm in 20 ml
and the KOH will be deluted by a factor of 5 (10 ml KOH, 40 ml H_2O)

Jan 14 2019

Titration of protein via EC using 1gr/20ml = 20ml
~~8ml of 1.0M KOH + 6ml H_2O~~
8ml of 1.0M KOH + 12 ml H_2O. = 20ml

	t	vol	vol	EC	
*	1	18.75 mg		4.71	
	3	18.57		4.61	Color change immediately visible
	5	18.38		4.69	
	7	18.20		4.77	full green now
	9	17.95		4.89	dark green now
	11	17.38		5.16	
	12	16.60		5.50	
	14	15.44		6.01	
	16	14.85		6.48	
	18	14.59		6.72	
	19	14.41		6.87	
	21	14.28		7.01	
	23	13.90		7.34	
	25	13.34		7.80	
	27	12.54		8.54	
	28	12.02		9.03	stall point?
	31	11.60		9.38	
	33	11.04		9.89	
	35	10.59		10.33	
	37	9.90		11.00	
	39	8.82		12.06	
	41	8.02		12.90	
*	—	16.98 mg		—	
	44	16.05		13.64	
	47	14.84		14.58	

t	vol	vol	EC
57	13.11		15.85
52	11.45		11.18

Thermo never reached the second equivalency point. ??

Jan 15 2019

Of interest that no equivalency point was reached with a very weak protein solution. We do still have the short & small minimum that occurs almost immediately (~0.25ml) - the remaining interest must it should be H_1 of H_2A.

I surmise that concentrations must be increased but I must preserve the protein sample to the highest degree possible.

Recommend 2gm of protein in 20gm of (H2O + protein)
Recommend 0.1M KOH ⇒ 1ml KOH / 10ml(KOH+H2O) ⇒ 14ml KOH / 20ml(KOH+H2O)

t	vol	VOL	EC	t	vol	VOL	EC
0	19.33	0	7.47	51	14.80	4.53	10.34
1	19.32	.01	7.39	55	14.43	4.90	10.74
7	19.25	.08	7.29	56	13.86	5.47	11.35
16	19.20	.13	7.28 Color Shift	58	12.40	6.93	12.90
23	19.14	.19	7.29	60	11.41	7.92	13.96
26	19.08	.25	7.32	65	10.50	8.75	14.97
28	19.01	.26	7.35	68	7.86	7.86 ALT	18.00
29	18.84	.49	7.55 Green				
30	18.51	.82	7.19				
34	17.11	2.22	8.69				
40	16.57	2.76	9.03				
42	16.13	3.20	9.35 Stall appears				
43	16.05	3.28	9.32				
45	15.57	3.76	9.64				
49	15.24	4.09	9.94				

The titration is essentially linear across the entire range. $r^2 = 0.978$

I will return to pH titration.

Let's try 2gms protein w/ pH Titration in 20ml
@ 14ml KOH/20ml (H_2O + KOH):

t	Vol	Vol	pH	
0	18.10 ml	0	2.98	We have a very good
2	18.04	.03	3.04	titration run here
3	18.04	.06	3.14	w/ two dynamic
4	18.04	.06	3.23	equivalent points.
5	17.97	.13	3.44	This is an excellent
6	17.83	.27	6.02	example of a dynamic acid
8	17.68	.42	7.03	and the concentration
10	17.55	.55	7.35	are now correct.
12	17.28	.82	7.66	
13	16.66	1.44	8.18	Our first equivalent pt
15	15.76	2.34	8.52	$\tilde{=}$ 0.33 ml (pH $\tilde{=}$ 6.4)
19	14.33	3.77	9.12	
20	14.17	3.93	9.40	Our second pt $\tilde{=}$ 4.3 ml
21	14.02	4.08	9.97	KOH
22	13.83	4.27	10.45	1M NaOH = 56.11gms = X
23	13.66	4.44	10.77	1000ml 4.3ml
24	13.47	4.63	10.98	pH (~10.5)
25	13.31	4.79	11.14	X = 0.241 gms
26	12.77	5.33	11.48	
27	12.39	5.71	11.64	= .241gms = .0043 moles
27	11.04	7.06	12.01	56.11gms/mol
28	9.52	8.58	12.28	95% $\tilde{=}$ 46.5
28	7.84	10.26	12.50	90% $\tilde{=}$ 93.0
				85% $\tilde{=}$ 139.5

Assume 95% H_2O: ~~53.2 gms~~ ~~46.4~~

$MW \tilde{=} \left(\frac{0.05}{\text{Equation molar factor}}\right)\left[\frac{2\text{gms}}{.0043\text{ moles}}\right] = 463$ gms/mole; 90% H_2O = ~~53.2 gms/mol~~

~~95% H_2O = 34.88 gms/mol~~ ~~69.6 gms/mol~~

The next crucial piece of information required in the MW determination is the amount of water in the sample.

This will take some time as we are in a cloudy season and several days ⇔ weeks will be required to dessicate the sample. We have an estimated range from ~~23-70~~ 46-140 gms/mole @ the time. (Set: ~~23, 46, 70~~ 46, 93, 140) (95%, 90%, 85%) $\frac{\text{gms}}{\text{mol}}$

The next question is to study the half points, K_p, K_a, etc to see what they can tell us. Can your equivalency points be used to help identify the acid involved?

Some examples of diprotic acids:

		MW
H_2SO_4	sulfuric acid	98.1
H_2CO_3	Carbonic acid	62.0
H_2S	Hydrogen sulfide	34.1
H_2CrO_4	Chromic acid	118.0
$H_2Cr_2O_4$	Oxalic acid	170.0

The most likely candidates, @ least from the set are Carbonic acid and hydrogen sulfide. Could also easily be H_2SO_4.

$\frac{X(2)2}{1.0043}$

$$\frac{X(2\text{gms})}{\left[\frac{.0043\text{ mole}}{2}\right]} = 62.0 \text{ or } 34.1$$

$X_{62} = 93.3\%\ H_2O \stackrel{?}{=} H_2CO_3$

$X_{34} = 96.3\%\ H_2O \stackrel{?}{=} H_2S$

$$MW = \cancel{Dec\%}\left(\frac{\left(\frac{(1-Dec\%)(2gms)}{1}\right)}{\left(\frac{.0043 moles}{2}\right)}\right) = \frac{(1-Dec\%)(2)(2)}{.0043 moles}$$

$$\frac{MW(.0043 moles)}{4} = (1-Dec\%) \quad \text{or} \quad -Dec\% = \cancel{1-} \frac{MW(.0043 moles)}{4} - 1$$

$$\text{or } \underset{\text{Concentration } H_2O}{\underset{\text{imal}}{Dec\%}} = 1 - \frac{MW(.0043 moles)}{4}$$

H_2SO_4	MW: 98.1	⇒ Dec%	= ~~.105~~ .895	= 89.5% H_2O
H_2CO_3	62	⇒	= .933	93.3% H_2O
H_2S	34.	⇒	= .963	96.3% H_2O

This is the type of accuracy required in the evaporative process to determine the likely acid involved, assuming it is w/in this list.
There is no known source for chromium so @ this time the latter two acids are not candidates.

Are there known titration curves for each of these acids?
We know that we have two equivalence points at
~ pH = 6.4
~ pH = 10.5
known titration curves would be very helpful here.

The "half equivalence" point is when enough base has been added so that half of the acid is converted to the conjugate base. At this point:
H^+ concentration = Ka
and pH = pKa

$$pKa = -\log_{10}(Ka)$$

* SEE NOTES FOR JUNE 15 2019. OUR BEST DETERMINATION OF THE POLYPROTIC ACID IN THE SOLUBLE PROTEIN COMPLEX IS NOW PHOSPHORIC ACID

H_2S is a weak acid & foul smelling. At the time, the does not fit the bill.

* Every thing says that we have a strong acid (look @ pH of highly diluted protein). NOT SULFUROUS ACID

The strongly suggests that we are working w/ sulfuric acid here. Sulfurous Acid does not even readily exist.

The midpoint is therefore a very important point and looks to be easy to determine.

Our first midpoint should be

$(1/2)$ of $0.33\,ml \cong 0.165\,ml$

The pH $\cong 4$

Our second midpoint is halfway between the volume of the two equivalent points

$$= \frac{(0.33\,ml + 4.3\,ml)}{2} \cong 2.35\,ml$$

Our pH at the point $\cong 8.5$

since $pKa = -\log_{10}(Ka)$

~~$Ka = -pKa$~~ $10^{pKa} = -Ka$

or $Ka = -10^{-pKa}$

Note:
June 15, 2019: This work will be subject to review via improved titration method & pH meter calibration

Important Conclusion: Production of sulfurous acid.

At the midpoints, pH = pKa and -pH = -pKa
Therefore, at the midpoints

$Ka = 10^{-pH}$ or $K_1 = 10^{-4} = 1E-4$
$K_2 = 10^{-8.5} = 1E-8.5$

Look up K_1 & K_2 for H_2SO_4.

Well, guess what. Nutrients also support H_2SO_4

The Values for "sulfurous acid are"

K_1 1.4E-2 vs ~ $K_{1\,estimated} \approx 1E-4$
K_2 6.5 E-8 $K_{2\,estimated} \approx 1E-8.5$

NOT TRUE PHOSPHORIC ACID

And guess what? Nothing else comes close, eg

No C	Oxalic acid	5.6E-2	5.4E-5
Noodor	hydrogen sulfide	9E-8	1E-11
weak	Carbonic acid	4.5E-7	4.7E-11

We have some uncertainty in K_1 because we are not starting w/c pH of 0. We are starting with pH = 2.90 which is already greater than 10^{-2} to begin with.

This is another profound conclusion that results from the body of work over the past several weeks using the methods of titration & stoichiometry.

* The water soluble protein in addition to tying up vast amounts of iron to metabolize, grow & reproduce
  is also
  Producing a significant level of sulfurous acid.

And of course, the big question now is,

What is the result and impact from chronic exposure to dilute sulfuric acid?

What is the toxicity of sulfuric acid?
Low level exposure:

1. Respiratory irritation
2. Skin dryness, cracking
3. Non productive cough

Sound familiar, related, or associated?

Also see "Another Murder for Examination" research paper.

A mechanism for toxicity on the protozoa we quite applicable.

Toxicity effects of an acid metabolism?

Research on Cancer and acidity?

Jan 16 2019

Now that we tentatively know the specific acid that is involved we can state the reaction plus determine the concentration.

~~$H_2SO_4 + 2KOH \rightarrow 2H_2O + 2KSO_4$~~ Balance this.

$H_2SO_4 + 2KOH \rightarrow 2H_2O + 2K^+ + SO_4^-$ Balanced.

$M_1V_1 = M_2V_2$ M_1 = Titrand
M_2 = Titrant

$V_1 = 20ml$ $V_2 = 4.3ml$ KOH (1M) m = molarity, not gms.
$M_1 = \cancel{2gms}$ $M_2 = \cancel{9.30gms}$?
.02039 moles (not molarity

MW of H_2SO_4 = 98.08 gms/mole so $\frac{2gms}{98.08\ gm/mol}$ = .02039 moles not molarity

M_2 = .095 moles
but remember, 2 mole of KOH are ~~prod~~ used for every 1 mole of H_2SO_4.

M_1 is the concentration in molarity & M_2 is the molarity

for determining the molarity of M_1, this is what we do not know.
We do know the molarity of the KOH
$M_1 \cdot 20ml = (1M)(\text{undiluted}) \cdot 4.3ml \left(\frac{14ml\ 1M\ KOH}{20ml\ (KOH + H_2O)}\right)$ ← dilution ratio.

Therefore M_1 = Ø.15 molar H_2SO_4

$1M\ H_2SO_4 = \frac{98.08gms}{1000ml}$ & $.15M = \frac{14.712gms\ H_2SO_4}{1000ml\ (H_2SO_4)} = \frac{1.47gms}{100ml}$

for a 5gm sample, 1.5% = .075 gms calculated = 98.53% H_2O = 1.47%

Jan 18 2019

Two goals on the horizon:

1. Seek an independent verification of the existence of SO_4^{2-}

2. Begin the titration and comparison of kombucha - a closely related compound.
— A Homebrew

Let's start w/ 5 mL of kombucha in 20 mL of H_2O.
Done. Titrate w/ 1M KOH:

t	Vol	VOL	pH	
0	18.63	0	3.40	There is a very defined titration curve. It appears to be monoprotic @ this point.
1	18.59	.04	3.74	
2	18.54	.09	4.01	
3	18.48	.15	4.25	
4	18.42	.21	4.39	
4	18.36	.27	4.55	
5	18.22	.41	5.01	There is a very minor inflection point @ 0.55 mL.
6	17.93	.70	6.33	
6.7	17.85	.78	9.02 * slight color change	
8	17.73	.90	10.61	
9	17.60	1.03	11.40	Our primary inflection point is @ ~ 1.45 mL
10	17.32	1.31	11.88	
11	16.76	1.87	12.30	
12	15.66	2.97	12.72	
12	14.21	4.42	12.97	
13	7.81	10.82	13.31	No major color changes

Our equivalency point appears to be @ ~ 9.0 pH.
w/ ~ 0.90 ml

Let's see if we can estimate the molecular weight.

$$1M\ KOH = \frac{56.11\ gms}{1000\ ml} \Leftrightarrow \frac{x}{0.90\ ml} \qquad x = .050\ gms \cong .0505\ gms$$

$$\frac{.0505\ gms}{56.11\ gms/mol} = .00090\ mole\ KOH$$

We assume our mole ratio is 1:1 @ this point.
This means we have .0090 mole HA in the Kombucha.
Now unless you know the water content of the titrand (analyte) you cannot determine how many grams of titrant you have placed into solution.

Determining the Ka seems to be the next most important step.
The midpoint is 1/2 of the volume of a monoprotic titration, in this case 0.90 ml / 2 = 0.45 ml
This corresponds to a titration pt on our curve w/ a pH of ~ 5.0

This means Ka of our acid $\cong$ $Ka = 10^{-pH} = 1E-5$
We therefore surmise that we are looking for a monoprotic acid w/ Ka $\cong$ 1E-5
Given what the Ka is for acetic acid is 1.8E-5,
This would be right on track w/ expectations

Now let's look @ a molecular weight estimate
Acetic acid = CH_3COOH MW = 60.05 gms/mol

Now you still cannot determine the MW w/o knowing the amount of water involved, however if we assume we have roughly ~5% by weight we have

$\approx$ (4 ml vinegar)(.05 dec) = 0.200 ml $\approx$ 0.200 gms

The 0.200 gms is now diluted in 20 ml of H_2O.

As a rough estimate, the MW = $\frac{0.219 \text{ gms}}{.00090 \text{ moles}}$ = ~~220~~ 222 gms/mole

but the actual vinegar MW is 60.05 so the data not matched well.

Carbonic acid has a K_a of $4.5E^{-7}$ & $4.7E^{-11}$

Not a good match here either.
The is inaccurate.

Well guess what. Our sample is hardly pure vinegar.

It was 1/2 Cup vinegar / ~2.5 Cups total = 1/2 Cup Vinegar / (1/2 cup vinegar + 2.5 cups H_2O) = .05 / 3 cups total

= .167

So our expected MW = .167 (222 gms/mol) 37 gms/mol vs 60 gms/mol

Very rough estimate only here.

We are definitely in range.

All signs point to the existence of acetic acid (vinegar) o/n the Kombucha.

So we are definitely working w/a different animal here, even though the IR spectrum has an amazing similarity. But we can see that the acids involved between the protein and the Kombucha are entirely different from one another.

Jan 21 2019

Comments first:

1. It is now time to weigh the separated protein. We now conclude, a priori, that the sample is not composed of an acid alone, but is a complex mixture of coordinated iron complexes (Fe^{+3}), aldehydes, acid (sulfonic postulated) and proteins.

   Nevertheless, we shall weigh the evaporated sample to eliminate the water content.

2. We need an independent test run for SO_4^{2-} in the sample to additionally verify H_2SO_4

3. The recent ultrasound treatment on my upper lung & right shoulder have produced remarkably beneficial results. Ultrasound continues to rank as a therapeutic protocol, presumably degrading lung slime and microbial growth in these body regions.

4. I have had a professional ultrasound assessment. I generally hold come out well on the (primarily) torso area examined (primarily organ examination).

I do have two issues showing up:

1. The presence of a 2.5 cm (~1 inch) nodule (cyst) in the thyroid on my right side.

We will reexamine potential therapies. These will include a resumption of iodine intake and (possible) ultrasound? (May be hazards w/ thyroid).

2. I also am likely to have gall stones. Am non-symptomatic.

90-95% of thyroid nodules are benign, but this also means that 5-10% are not. Also ~ 50-60% of males over 60 have such nodules. The size of mine does seem to warrant some concern.

Also, Plaque formation in the left femoral artery is estimated @ 10%. 20% is the flagged level and 70% is the surgery level.

We now have another objective ahead of us. It is to determine if we truly can now differentiate the likely identity of an unknown acid using the K_a values. We now presume that we can since there are many cases that we cannot determine the weight of the unknown solid acid in a solution. We will now state the titration and calculation methods that I have developed.

Let us weigh the largely evaporated protein sample:

The remaining mass of the protein + the sample container is 9.23 gms.

—

Ok, the initial data was recorded on Jan 13, 2019. The given we almost 1 week of evaporation.

Weigh boat = 5.00 gms

So our remaining protein sample now weighs 9.23
−5.00
= 4.23 gms

The original mass of the protein prior to evaporation is 6.45 gms
Therefore 6.45 − 4.23 gms = 2.20 gms of H_2O (or any other volatile) has evaporated.

Our first estimate on H_2O % is therefore $\frac{2.20}{6.45}$ H_2O = 34.1%

This is much lower than I expected.
We therefore do indeed have a highly concentrated protein that is combined w/ other compounds.

Our purity of the sample is therefore currently estimated at ~ $\frac{4.23 \text{ gms}}{6.45 \text{ gms}}$ = 65.6 ≈ 66% Pure CDB production
Therefore our solution is indeed highly concentrated.

Now, going back to the work of Jan 15, 2019

IF (and it is not)! and only if the entire problem sample was pure H_2SO_4, our estimate from the method of Jan 15 produces a molecular weight estimate of

$$0.66 = 1 - \frac{MW(.0043)}{4} \quad \text{or} \quad (.66-1)(4) = -MW(.0043)$$

$$\text{or MW weight estimate (if pure acid)} = \frac{(-4)(.66-1)}{.0043} \cong 316 \frac{\text{gms}}{\text{mole}}$$

However!! AS NOTED - the sample IS NOT PURE H_2SO_4 so the method is not viable.
The method of Ka determination DOES remain viable.
If we knew the % of H_2SO_4 then we could reverse our estimate for the molecular weight.

Since we do now presume the acid to be H_2SO_4 We can actually estimate the % of H_2SO_4 of the sample by:

$$\frac{98.1 \text{ gms/mole (MW of } H_2SO_4\text{)} - \text{presumed identified}}{316.3 \text{ gms/mole}} = 31\% \ H_2SO_4$$

presumed concentration of H_2SO_4 @ the point of examination.

We will now proceed to Ka determination of "Unknown" acids, for control and reference purposes.

Jan 30 2019

Let's proceed toward identification of an "unknown" acid.

I will use ascorbic acid as an example and assume that I do not know it's identity but attempt to determine the molecular weight.

We know we need to determine the equivalence point(s) — we also need to learn if it is monoprotic or polyprotic along the way. We know that the midpoints are crucial points.

I am allowed to use previous titrations to identify a functional titration.

Citric acid is $C_6H_8O_7$
Ascorbic acid is $C_6H_8O_6$ M 176.12 gms/mol

We have a titration trial on Jan 10 2019.

Notice that we take the question of volume used came up. We also only know the mass %, not the molar concentration.

This was actually an interesting case as we know we used the final titration volume of 27 mL vs the original volume of 20 mL.

The question of mass % is an interesting question. Let's use hand look @ the data and try to go in reverse.

The data is:
We started w/ 20 ml of a 4.91% by mass of ascorbic acid.
The equivalence point was reached @ 7.25 ml with the use of 1.0M KOH

An unknown factor is that we do not know if citric acid is monoprotic or polyprotic

$MW = \frac{gms}{moles}$ or (Moles = $\frac{gms}{MW}$ or ie $\frac{40 gms}{40 gms/mol} = 1 mol$) MW can be derived from this

Define a mole: 1 mole = No. of grams in a mole / gms/mole

so a mole is unitless.

Citric acid is monoprotic but we would not know this.
No of mole KOH: $\frac{56.11 gms}{1000 ml} = \frac{X}{7.25 ml}$ $X = .407$ gms

$\frac{.407 gms}{56.11 gms/mol} = .00725$ moles

So assuming a monoprotic acid (as supported by the titration result above) we know that we have

.407 moles of a 4.91% mass solution (20 ml) that required the addition of 7.25 ml to reach the equivalence point.

Now the interesting question to me is that the 4.91% mass solution in 20 ml is no longer 4.91% by mass WHEN the equivalency point is reached.

Now it becomes

$\frac{4.91\%}{20\,ml}$ ⇒ but it is diluted now by the ratio

NO! of $\frac{20\,ml}{27\,ml} = 0.741$ This means that at the point of equivalency it becomes a $4.91\%(.741) = 3.64\%$

NO! So this says we have a 3.64% mass solution

$\frac{3.64\,gms}{100\,ml} = \frac{x}{27\,ml} = 0.983$ gms of the unknown acid in 27 ml

NO $MW = \frac{gms}{moles} = \frac{.983\,gms}{.00725\,moles} = 135.6$ is our MW estimate.

Actual is 176.12, so what happened here.

The problem is we do need to use the original concentration of the citric acid, not the diluted

Our problem set up is

$\frac{4.91\,gms}{100\,ml} = \frac{x\,gms}{27\,ml}$ $x = 1.326$ gms.

OK $MW = \frac{1.326\,gms}{.00725\,moles} = \frac{183\,gms}{mol}$ vs actual $\frac{176.1\,gms}{mol}$

Therefore, you use the original concentration of mass %, not the diluted solution.

We therefore look for a monoprotic acid w/ a molecular weight ~~alternate~~ of $\frac{183 gms}{mole} \pm 5\%$

Now in addition, can we form an ~~alternate~~ for Ka?

The mid point will be @ (7.25/2) mL = 3.625 mL
The corresponds to a pH of ~ 4.1

$Ka = 10^{-pH} = 10^{-4.1}$ or $Ka \approx 1 * 10^{-4}$

What is the Ka of ascorbic acid?

8E-5

The is off only by a factor of 1.25 so the is decent

Monoprotic

Our unknown acid has a MW of $\frac{183 gms}{mol} \pm 5\%$

What acid fits the bill?

a Ka of ≈ 1E-4

$pKa = -\log Ka$

$pKa = -\log(1E-4) = +4$

So $Ka = 10^{-pKa}$ ≈ ~~0~~

There should not be many acids that fill the bill
Is it likely that any other acid fit the determination?

Our pH software is our first and foremost allie here.

We notice it has 43 acids in the database. It is fascinating but it shows ascorbic acid as actually being a dyprotic acid.

However, notice that pKa, is indeed 4.2

vs our estimate of 4.1

so already we have a candidate for identification of ascorbic acid.

Meas	Additional Candidates based upon pKa alone are:			Meas
4.1	Benzoic acid	4.2	122.1	
4.1	Succinic acid	4.2	163.4	
4.1	ascorbic acid	4.2	176.1	183 gms/mol

Closest candidate is indeed ascrobic acid.

Our software will fit the purpose and need.

The method worked marvelously well.

Further confirmation of H_2SO_4 existence in the soluble proteins

Test for sulfates:

$BaCl_2$ + dilute HCl produces a white precipitate in the presence of sulfate ions.

$BaOH_2 + HCl \rightarrow BaCl_2 + H_2O$

sulfate ions are not detected.

Do we have $BaCl_2$? The answer is highly fortunate since I happen to be on the road for four months.

* The result for the test for existence of sulfate ions is positive.

This further confirms the existence of H_2SO_4 in the protein.

NOTE SEE JUN 15 2019

* Our confirmation of H_2SO_4 is based upon:

1. A strong acid existence, at a low pH (~3.5)
2. The existence of a diprotic acid, as determined by titration.
3. The elements known to exist in the culture medium.
4. The determination of both Ka's for the acid via titration and the matching of those values w/ those of H_2SO_4.
5. The detection of sulfate ions, some of which will occur through dissociation of H_2SO_4 via a $BaCl_2$ + Dilute HCl chemical test.

SEE JUN 15 2019 NOTES

Some Foundation projects: Short Term Goals

1. Fund the formation of the foundation via legal support

2. Fund the writing of a book for public comprehension of CF research

3. Fund the DNA & protein testing

4. Fund the moderation of CHPN

5. Fund the construction of the website archive and the laboratory bulletin board

6. Transfer the recent laboratory accomplishments to the foundation to promote & sponsor more sophisticated research. (ie DNA, proteins, culture)

7. Fund the website maintenance and further development

8. Fund a second generation documentary.

Self-Assessment Comments:

* Exploratory Ultrasound Therapy is underway. It is thought that the

1. Thyroid nodule
2. The ~~chronic~~ Chronic low level cough, aggravated under conditions of weakness or fatigue (upper right lung)
3. The shoulder chronic low level pain and pain upon motion
4. The excessive production of daily ear wax frequently interfering w/ hearing ability

are all tied into the same causal condition. The Cross-domain microbial impacts are most definitely hypothesized to be at the root of this symptom set.

The recent identification of the filament-protein-biofilm network in the sputum sample during a severe respiratory condition (2 months required to ~~at~~ reduce the primary symptom set - frequently referred to as a "flu" (not true)) adds to the evidence for the hypothesis.

It is believed thought that a resident filament-protein-biofilm network exists in the upper right quadrant of my body: throat, thyroid, upper right lung, ear & drainage system, & the right shoulder.

The symptom set has been gradual and under long term development, actually over the last 20-25 years, first starting w/ the universal illness symptom set in Montana circa 1994.

Ultrasound & Methyl salicylate are my 2 most promising therapies at this point, along w/ all information presented in the "A Supplemental Discussion" paper (which is extensive in its own right). Supplements seemed to be also a mainstay of necessity by the therapy.

The "situation" has been under "management" for an extended period & portion of my life - CI realized is intrinsically tied in w/ this "management". At this point there has never been a complete eradication of all symptoms from my body. They have, however, been "managed" successfully now over the same extensive time period.

Feb 01 2019

Today

I have run a test w/ H_2SO_4 to test dissociation in distilled H_2O and the detection of sulfates.

The test succeeds on both accounts. Sulfates are indeed detected in dilute H_2SO_4.

In the case of our protein, the contents to support the presence of H_2SO_4. The would indicate that SO_4 from $FeSO_4$ & H_2O are used by the CDB to produce H_2SO_4. It is possible that you may also be detecting the original sulfates from $FeSO_4$ introduction, however the titration results clearly support the existence of H_2SO_4. It is possible to have two sources of SO_4, $FeSO_4$ & H_2SO_4. The will depend on the rate of conversion to H_2SO_4.

—

Another topic has emerged. I need to seriously investigate the presence of an alcohol. Qualitative alcohol test is desired since IR is not available here. Also need to review existing recent IR work along w/ the separation from recent liquid Chromatography (LC).

An article in Spectroscopy discusses NH vs OH bonds & the resulting IR absorbance - let us say that OH is closer on the table after studying the article along w/ my recollection of IR spectra.

It is also quite possible (and now somewhat expected) that both functional groups are present. Start w/ an OH qualitative test - how is the affected by evaporation & condensation of the protein?

Tests to distinguish between alcohols will be a bit more involved because of differences between primary, secondary & tertiary alcohols.

Phenols can be separated from alcohols by the use of ferric chloride, which we do have.

IR review looks like the best approach.

Also NIR – OH detection possible?

The does look possible.

~925 nm ROH Also ~725 nm!

~950 nm ArOH (also ~~725~~ 720 nm)

This may be possible w/ the Pasco spectrometer.

The Pasco spectrometer has a range of 380–950 nm. They say it should be possible to detect ROH w/ it.

Feb 02 2019

I have excellent NIR spectra taken w/ the Pasco VIS spectrometer: Range 300–950 nm.

1. Reference spectrum taken w/ distilled water
2. Reference spectrum taken with 2 drops of denatured alcohol in distilled water.
3. Sample spectrum taken w/ 2 drops of the soluble protein in distilled water.

The reference spectrum of denatured alcohol shows 3 absorption peaks (a region):

1. 386 nm
2. 447 nm
3. Increasing (monotonic) absorption from ~900–950 nm w/ max achieved @ 950 nm (end of range and no discrete singular peak). Clearly significant absorption, however, in the range of 900–950 nm.

The soluble protein (2 drops in distilled water) shows similar magnitude absorption @:

1. 406 nm
2. 445 nm
3. 946 nm peak absorption obtained.

The means that the protein does clearly show the existence of an alcohol, which was now the emphasis of the study of the NH vs OH bond - IR spectra discussion in the current issue of Spectroscopy magazine.

Furthermore, the result indicate the likely presence of ArOH

which is even more interesting and significant. Previous tests indicated a failure for phenol existence.

The will now all be repeated.

What are other various examples of ArOH?
What are their general properties?
How might we learn more about what form of NaOH is in question here.

The most certainly does not eliminate the existence of the NH bond (essentially guaranteed by the positive protein tests) however further scrutiny is to be made here between alcohols, amines, and protein.

Very good and usable results by the spectrometer approach here.

Feb 03 2019

A major ultrasound (US) investigative session late last night.

US of varying duration & strength was applied to four different regions of activity.

1. First, the intestinal area. I have occasional intestinal blockage. It is also known (slowly over the years) that an allergy to red pepper induces this. Eating too much seems to cause the also - system can simply not process digestion as easily @ this stage.

Main observation (and this has occurred twice now) is that US definitely seems to provide relief in the area. & also combined w/ the use of enzymes w/ this strategy - the combinations seem to be quite helpful.

2. The chronic central point of pain in the right shoulder is of much interest. The high power setting used a few days ago, definitely induced a deep, sharp specific location pain. It may or may not be @ the level of the bone. I backed off to medium power and no deep sharp pain was felt afterwards in the session.

In the case, med power was first used w/ no pain present. High power was also almost completely w/out pain, but eventually the point was located again. The pain level was much lower than after the first session under high power.

This is a very promising occurrence. The shoulder issue is a central health issue, and has been a problem for many years as now.

US seems to hold some promise of the centralized location can continue to be reduced in pain.

3. The upper right chest region (location of the chronic low level cough) was US treated at mid-level power for ~10 min. Only beneficial results have been observed from the strategy.

4. The thyroid on the right side (location of 2.5 cm nodule-cyst) was treated for 5 min @ low power.

This is a delicate & higher risk issue that is under careful monitoring for any physiological differences.

This was a very busy & comprehensive US session.

Feb 05 2019

Let's take stock of some of the components we know are in the protein (soluble)

1. We know now that we have sulfuric acid
2. Iron is known
   1. Is it in ionic form?
   2. Color says we have Fe^{+3}. brown
   3. We know that we have a coordination complex formed upon exposure to bleach
3. Sulfates known (here H_2SO_4 or $FeSO_4$ are candidates)
4. Peptides are known
5. Interest to test for both amines & ionic forms of iron.
5-5 Phosphate ions $H_2PO_4^{2-}$ (See Feb 9 2019 notes)
6. We learn that the results of "mild oxidation" are:
   1. alcohols (during combustion of hydrocarbons)
   2. aldehydes
   3. acids

NOTE

6-5 Nitrate ions NO_3^- also appear to be present (Feb 09 2019)
7. I have demonstrated the presence of an alcohol
8. I have demonstrated the presence of an aldehyde
9. The presence of thiocyanates confirmed & to be established

10. Titration has been used to determine the identity of H_2SO_4.
11. Elemental/ionic identification by electro chemistry would be valuable.

12. Liquid Chromatography has been used to separate important constituents in combination w/ IR
13. Thiocyanates demonstrated in previous tests; Research paper written.

Next most valuable move is most likely to use the data combination from both infrared & liquid chromatography.

A significant session of work of LC-IR took place starting Dec 22 2019.

Before we get into the examination, there is a fundamental flow chart w.r.t. oxidation of hydrocarbons.

A fundamental sequence is that the "mild oxidation" of hydrocarbons produces

1. Alcohols
2. Aldehydes
3. Organic acids

} and not only that, but in the sequence.

Mild oxidation results from the insertion of an oxygen or the removal of two hydrogen from the molecule.

Ex methane (a classic hydrocarbon):

Methane + Oxygen $\xrightarrow{\text{leads to}}$ Methyl Alcohol $- H_2 \xrightarrow{\text{leads to}}$ Formaldehyde

+ Oxygen $\xrightarrow{\text{leads to}}$ Formic Acid + Oxygen $\xrightarrow{\text{leads to}}$ Complete Combustion $H_2O + CO_2$

*

Fundamental Flowchart Sequence of Organic Chemistry

This is really quite profound, as it shows a definite sequence in the creation of functional groups w/in organic chemistry.

Now you can see how important a fundamental:

1. Hydrocarbons
2. Alcohols
3. Aldehydes
4. Organic Acids

In addition, these two combine to create esters

are:

and ethers are formed by the dehydation of alcohols w/ sulfuric acid

Therefore, a major aspect of organic chemistry derives from this flowchart alone

* and we already have the majority of these compounds already discovered within our soluble protein, and this certainly tells us that we have such intermediate

* oxidation products being formed from source hydrocarbons.

This really places a lot of organic chemistry into context from this basic understanding.

Now let's start working w/ the LC - IR work. Dec 22
These sessions were incredibly productive and they show the power of combining LC w/ IR.
I have many sequenced spectra to review here.

Tube 1-5 was our first separation observed, isolated w/ a neutral ORP and a pH of ~7.4. The method was one of applying a strong base to the column, which we know contains a soluble, highly acidic CDB produced protein.

We see four peaks and a surprising absence of hydrocarbons.

The peaks are @: (w/ ATR used) in order of magnitude

1. 1102 cm^{-1} very strong, narrow (~1200-1000 cm^{-1})
Candidates.
2. 613 cm^{-1} strong, presumed narrow

3. 1351 cm^{-1} weak, wide

4. 1670 cm^{-1} weak

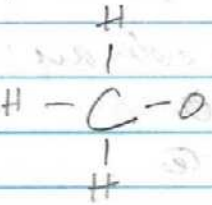
Closest SDBS match is C_3H_7NO
ethyl foramide MW 73.1

Not a good match.

Let's look @ the 1102 cm^{-1} candidates.
Notice it's extremely strong, w/ a range of ~1200 - 1000 cm^{-1}.
Our main identification reference is Parker table.

It's of interest that alcohols fit this rely well, ie 1200 - 1000 cm^{-1} range, strong peak.
The question is, why no hydrocarbons showing up at all in the plot? What is the simplest alcohol?

Methanol is.



Two alcohol types are listed by Parker @ 1102 cm^{-1}.

1124 - 1087 cm^{-1} Secondary open-chained saturated.
1124 - 1087 cm^{-1} Unsaturated or cyclic tertiary.

Methanol does indeed have strong absorption @ ~1080 cm^{-1} and also broader weaker absorption near ~1400 cm^{-1} but some enough hydrocarbons are strong w/ the methyl group. With the exception of the hydrocarbon issue, we do have indication of a possible alcohol here.

Now things get a lot more interesting.

I have tightened up the parameters of search on SDBS.

Now I use the 1102 + 613 cm^{-1} peaks w/ a min of 30% or transmittance

a clear category is showing up.

The first six and all six lookups are of

SULFATES.

The closest matches in order are:
methylamine sulfate
+ ethyl amine sulfate.

The lack of HC absorption is not so surprising now in consideration of ATR. Peaks in this region are always weaker w/ ATR and they may simply be too weak to detect on the particular sample run.

What is also fascinating is that methylamine sulfate is also shown as being in combination w/ sulfuric acid, ie

$CH_5N \cdot \frac{1}{2} H_2SO_4$

$CH_3-NH_2 \cdot \frac{1}{2} H_2SO_4$

Even though methyl amine sulfate is actually only CH_5N

I would say that we have apparently built almost certainly a sulfate compound.

The H_2SO_4 connection is interesting.
The amine peaks appear to be quite strong in the SDBS spectrum. We do not see them and they simply may not be present in the particular sample.

In Parker, SO_4^{2-} is indeed a very good match @ ~ 1105 cm^{-1}. Also the pH is listed @ 7.4 and we are @ pH conditions of 7.4.

The 613 cm^{-1} peak remains ambiguous for us. Parker has a SO_3^{2-} peak @ ~ 780 cm^{-1}, taken as the closest most reasonable history.

From Parker, general table, the closest in the range is bromo derivatives (halogen) @ ~650.

Interestingly, however, that SDBS does show strong absorption @ ~ 613 cm^{-1} with the methylamine sulfate, ie $CH_3NH_2 \cdot 1/2\ H_2SO_4$.

What we can say w/ a high degree of confidence is that we have isolated a sulfate compound. The specifics of that sulfate compound are not identified further @ this point.

I have now extended the search to include the 1357 cm^{-1} peak w/ max absorbance @ 50%.

Our closest match here is amide amin- amidinourea sulfate.

$C_2H_6N_4O$ hemi w/ Lewis also shown w/ H_2SO_4:

$$NH_2-\underset{\underset{NH}{\|}}{C}-NH-\underset{\underset{O}{\|}}{C}-NH_2 \cdot \tfrac{1}{2} H_2SO_4 \cdot H_2O$$

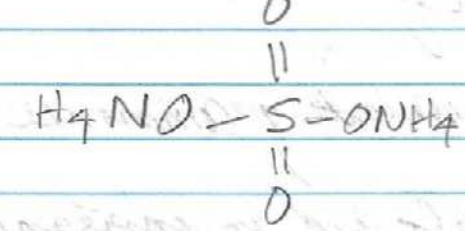
Notice both compounds of closest match remain as sulfates and contain amines.

Looking @ the substructure more broadly, this would suggest a category compound of "amine sulfates".

Investigating this further, a top candidate of investigation will that of

Ammonium sulfate $H_8N_2O_4S$ w/ MW 132.2

Now, if we look @ the IR spectrum of the compound which fortunately has also been found also w/a SDBS database.



The spectrum for this is amazingly a match, w/ the exception of a peak @ ~ 3130 cm^{-1}.

We have matching peaks @
~ 1100
~ 615
~ 1400

The looks to be our match.

I would say that Ammonium sulfate has been isolated via the methods of LC & IR.

Now, for the interpretation, toxicity —

Causes irritation to the respiratory tract.
Causes irritation to the gastrointestinal track.
Causes irritation to skin.
Widely used as a fertilizer.
The source of nitrogen here would as such be the acid.

And guess what?

Ammonium sulfate is an inorganic salt obtained by the reaction of

SULFURIC ACID w/ 2 equivalents of ammonia.

It is an ammonium salt and an inorganic sulfate salt.

Looks like we have a rather strong match of circumstances.

Feb 09 2017

A few more comments on the IR spectrum under review (ie, sulphate compound)

The analysis of determination of a sulphate compound seems to be absolutely correct.

The question of ammonium sulphate remains somewhat uncertain however due to the lack of the 3130 cm^{-1} peak. ATR weakness @ high cm^{-1} may be a contributing factor, cannot say.

We do know that IR spectra of inorganic sulphate compounds is baffling even many IR experienced spectroscopists!

A couple of posts identify a similar IR spectrum, esp the strong 1102 peak w/out any CH peaks as sodium sulphate. A ferrous sulphate IR plot also shows the strong peak @ ~1100, the med. peaks @ ~600 and 1600 cm^{-1}, so these are also on the right track.

I think what can be said w/ certainty is that we do indeed have a sulphate compound. Furthermore, it is likely an inorganic sulphate compound but it is difficult to be more certain than that. Ammonium sulphate, sodium sulphate and ferrous sulphate would all be possibilities (for example. (ferric also?)

* Common Ground

Proteins, Pathology, Alzheimer's & Morgellons —

* The article on Alzheimer's is presented on the following page for a very important reason.

* It portrays, on a dramatic scale, the importance of knowledge of proteins in pathology.

* What has happened here is that Alzheimer's can now be detected in the blood, via the brain. This is a MAJOR advancement.

* This has occurred because of an increased knowledge in the detection of the specific proteins, now in blood.

* This has taken decades of research to accomplish.

* The case of "Morgellons" shall be no different. The detailed knowledge of the proteins will be an essential part of the process.

* The stage for ACCOMPLISHING this is now in place. I may never see such a result in my lifetime but the path of pursuit is quite clear now.

* We also have a very important statement:
"Many diseases, including those of the heart, liver & kidney, CAN BE DIAGNOSED BY THE PRESENCE of higher than normal CONCENTRATIONS OF PARTICULAR PROTEINS IN BLOOD SERUM"

Chemistry, An Integrated Approach by Kenneth Raymond 2008 pg 59



Researchers say a reliable blood test for Alzheimer's disease is in sight.

DIAGNOSTICS

A blood test for Alzheimer's disease draws near

New assays and biomarkers are bringing the decades-long quest closer to the finish line

JYOTI MADHUSOODANAN, special to C&EN

Years ago, Sid O'Bryant learned that his grandmother was showing signs of a failing memory: she often got lost driving and needed notes posted around the house reminding herself to take medications. O'Bryant was a medical student then and had worked with people who had similar symptoms. But despite his experience and network of connections to medical experts, he found it tough to help his family get a firm diagnosis for his grandmother's illness. Eventually, doctors concluded it was Alzheimer's disease (AD), O'Bryant says, but the effort to reach that answer was a huge source of stress for the family.

Her situation is still frustratingly common. Approximately 5.5 million Americans over the age of 65 cope with these and similar indicators of AD. Symptoms include behavioral changes, cognitive difficulties, and mood swings: these could indicate a number of different illnesses, so many people don't know for sure what they are battling.

For the vast majority of these people, including O'Bryant's grandmother, no single test can provide a diagnosis. Physicians use a combination of cognitive tests, family medical history, and neurologic exams to evaluate a person's mental function. Blood tests and magnetic resonance imaging (MRI) scans to image the brain can only rule out other illnesses such as tumors or vitamin deficiencies. This means that in many cases, a diagnosis of AD is one of exclusion—there's nothing else left it could be. In the hands of specialists, these methods yield accurate results "75 to 85% of the time," says neurologist Douglas Galasko of the University of California San Diego, "but many patients with AD never see a specialist, so error rates may be much higher, mainly with the disease being underdetected."

Better diagnostic tests do exist. Positron emission tomography (PET) scans can reveal the abnormal protein clusters in people's brains that are a hallmark of the disease, and lumbar punctures—needles inserted into the spinal canal under local anesthesia—can detect these proteins in the cerebrospinal fluid (CSF). These tests can definitively diagnose AD, but they are also invasive, cost thousands of dollars, and are not covered by most health insurance plans in the US. So most people never get tested.

O'Bryant, driven in part by his grandmother's experience, is now a professor of pharmacology and neuroscience at the University of North Texas Health Science Center. He recalls submitting a grant seeking funds to develop a blood test for AD more than a decade ago. "I was told that one, we don't need a blood test, and two, it'll never work," he says.

Yet without such a simple test, it's difficult to catch AD early—a critical step in managing the disease with drugs and lifestyle changes to cope with problematic symptoms. It's also tough to identify people who could potentially participate in clinical

CREDIT: ROMASET/SHUTTERSTOCK

trials. Perhaps most importantly, a blood test, even an imperfect one, would be an important first screen for primary care doctors to figure out whether to refer someone like O'Bryant's grandmother to a specialist for more involved confirmatory tests.

After a hunt that's lasted nearly 20 years, a wave of new research now promises a sea change. Researchers have identified reliable, blood-based biomarkers of disease, thanks to more automated and sensitive detection techniques. "The improvement in methods makes it possible to learn much more about the biology" of the disease—and to develop a blood test to help patients, says neurochemist Henrik Zetterberg of the University of Gothenburg.

Better biomarkers

When AD was first discovered, the only way to be certain the disease was present was by performing an autopsy. A diseased brain shows clumps of abnormal amyloid protein between neurons and misfolded tangles of a protein known as tau inside the cells. Amyloid clumps, or plaques, are made of a mix of peptides of varying lengths. These peptides form when enzymes chop up a particular glycoprotein in the brain known as amyloid precursor protein (APP).

Researchers have known for decades that amyloid breakdown products slip out of the brain into CSF and then into the bloodstream. But quantifying their levels in blood proved nearly impossible for several reasons. For starters, the peptides' concentrations are low in blood. These small proteins are also sticky, clustering with other blood proteins and clinging to the surface of plastic collection tubes or other containers. So even simple factors such as the number of times a sample is frozen, thawed, or pipetted can alter results.

"People kept trying to measure amyloid peptides," even forming a global consortium to try to solve the problem, Zetterberg says. "But we couldn't reliably quantify and detect them, so the results were all over the place."

The breakthroughs began with a better understanding of specific biomarkers in the blood that reliably correlate with AD. Neurology researcher Randall J. Bateman of Washington University School of Medicine in St. Louis and his colleagues used radiolabeled amino acids to observe when and where amyloid protein was produced and cleared away in the brains of study participants (*Science* 2010, DOI: 10.1126/science.1197623). They found that, compared with the control group, people with AD had more amyloid-β 42 (Aβ42), an APP breakdown peptide that is strongly associated with AD, collecting between cells because it wasn't cleared away quickly enough.

> "I was told that one, we don't need a blood test, and two, it'll never work."
>
> **—Sid O'Bryant,** professor of pharmacology and neuroscience, University of North Texas Health Science Center

But its levels in the blood are not a reliable marker of AD, because several studies have found that amyloid peptides, including Aβ42, accumulate in the brains and blood of even cognitively normal adults as they grow older—and because half of circulating amyloid comes from other parts of the body, like the liver.

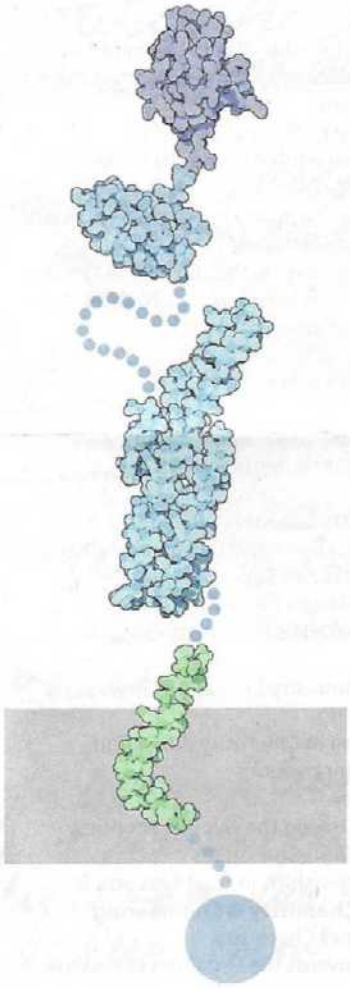
So early tests that aimed to measure the total amount of amyloid proteins in blood circulation proved unhelpful. But the mix of peptide fragments formed when APP is broken down offered a clue: some peptides are more strongly linked to disease-causing plaques than others. Now, a growing quorum of research shows that one particular marker—the ratio of Aβ42 to another amyloid breakdown peptide, Aβ40—might be the ideal basis for a blood test because this ratio reflects how the key brain enzyme digests APP differently in AD than in the general elderly population. Katsuhiko Yanagisawa of the National Center for Geriatrics and Gerontology and colleagues have found that with new techniques, the Aβ42-to-Aβ40 biomarker in blood is almost as accurate a test as amyloid and tau PET imaging (*Nature* 2018, DOI: 10.1038/nature25456). In follow-up work, the team tracked people with an abnormal Aβ42-to-Aβ40 ratio and found that within one to five years, several went on to have positive PET scans, suggesting the test can detect low levels of amyloid buildup early on. If such a test were to reach the clinic, people who appear to be at risk for AD could be monitored over time for further symptoms.

"When that ratio is abnormal, it's indicative of the presence of amyloid plaques," Bateman says. "The ratio really gives us the precision we need to individually identify people at risk."

Another classic marker of AD, tangles of the protein tau within neurons in the brain, has also proved tricky to measure accurately in the bloodstream; correlating those measurements with AD is also difficult. However, recent studies hint that tau, too, may yield useful information. Certain phosphorylated forms of tau in the plasma appear to stably correlate with signs of AD.

Yet another promising marker is a protein named neurofilament light (NFL). NFL is released from neurons damaged by injury, concussions, multiple sclerosis, or neurodegenerative conditions, including AD. Increasing nerve damage of any kind causes its levels in the blood to increase, so it's not specific to AD, but it is useful as a relative marker to track how a person's disease is progressing, says Michelle M. Mielke of the Mayo Clinic.

NFL is an "excellent candidate" biomarker that's further along in development for this purpose than the amyloid



Tangles of amyloid precursor protein (blue, membrane-spanning domain shown in green) in the brain are characteristic of Alzheimer's disease (AD). Derivatives of this protein may be part of a reliable blood test for AD.

CREDIT: DAVID S. GOODSELL/RCSB PDB/CC-BY-4.0

The Quanterix assay is 1,000-fold as sensitive as traditional enzyme-linked immunosorbent assays because it uses antibody-carrying magnetic beads that fit individually into femtoliter-volume wells. If a plasma protein of interest is present, the detection antibody linked to an enzyme binds to it. The enzyme goes on to cleave a chemiluminescent signaling molecule that can be detected in the well.

peptide ratio and tau markers that are specific to AD, Mielke says. But it's difficult to pinpoint a single marker as the best candidate for a blood test: "It depends o the context of use—some may be better for diagnosis and others to track disease progression or response to a drug."

In more of a shotgun approach, O'Bryant's team and others have developed panels of blood protein biomarkers purely according to their correlation to AD, regardless of their function: collective changes in the biomarkers' levels are linked to AD risk. Although this approach has yielded inconsistent results, particularly in trials with small numbers of patients O'Bryant is trying to rectify this with larg studies and a validated method. He is currently testing the team's panel with thousands of patients in primary care clinics.

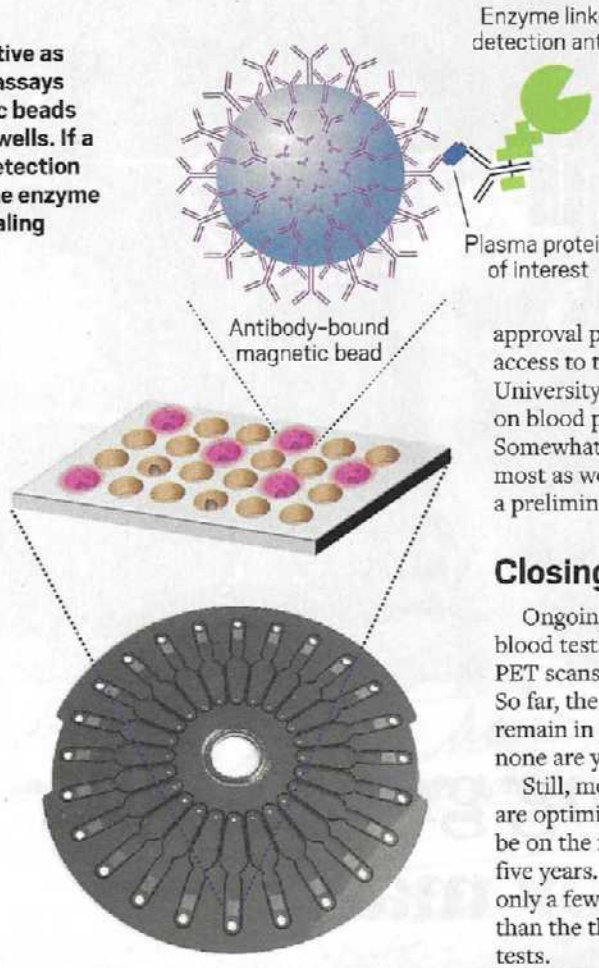
Even as researchers have homed in on better biomarkers, however, many agree that the key to achieving a blood test for AD lies in the methods used to measure these proteins.

Upgraded assays

Older tests to measure AD-related blood peptides lacked the sensitivity and precision required to distinguish a person with AD from someone without the disease. Since amyloid peptides also circulate in the blood of cognitively normal people as they age, the difference in the ratio of Aβ42 to Aβ40 between someone healthy and someone at risk of AD is small but significant, only about 15%, Bateman says.

New mass spectrometry instruments are now good enough to detect this difference. Just last year, three independent groups of researchers, including Bateman's group, used mass spectrometry to quantify amyloid peptides pulled from plasma using antibodies. One of these studies, for example, predicted disease risk on the basis of amyloid level with an accuracy of about 90% relative to those diagnosed by PET scan 12–60 months later.

Another new approach with groundbreaking sensitivity is a major upgrade to a classic technique known as enzyme-linked immunosorbent assay (ELISA). The Massachusetts-based firm Quanterix has developed an automated version of ELISA



Sample plate. One bead per 50 fL well, 200,000 wells per plate.

that is 1,000 times as sensitive as older systems. Instead of tethering antibodies to the floor of wells in a microtiter plate, which usually holds multiple antibodies in each well, the company's assay uses antibodies tethered to individual beads, which come to rest in individual wells on a grid. Each well is only 50 fL—about half a billionth the volume of a microtiter plate well—and holds only a single bead. By diluting plasma samples and adjusting the ratio of beads to protein molecules, researchers can ensure that each bead carries only one bound protein. An automated digital detection system measures the glow that results from a chemiluminescent reaction that occurs when the protein is present, allowing the assay to detect single protein molecules. These adjustments to dilution, reaction volumes, and standardization—coupled with antibodies that are highly specific to the peptide biomarkers—overcome problems including amyloid stickiness and ensure that newer assays outperform older attempts.

Others are homing in on biomarkers using similar automated ELISA-based approaches, such as Roche's Elecsys system, to detect amyloid peptides and phosphorylated tau proteins in CSF. Earlier this year, the US Food and Drug Administration granted the Elecsys assay a breakthrough device designation, which aims to expedite the approval process so people have quicker access to the test. Oskar Hansson of Lund University recently tested its performance on blood plasma samples instead of CSF. Somewhat to his surprise, it performed almost as well. "This could make it useful as a preliminary screen," Hansson says.

Closing in on the clinic

Ongoing studies aim to assess which blood tests measure up to the accuracy of PET scans and tests of CSF biomarkers. So far, the plethora of possible blood tests remain in preclinical and clinical studies; none are yet approved for commercial use.

Still, most researchers studying the area are optimistic that a blood test for AD will be on the market within approximately five years. A blood test is expected to cost only a few hundred dollars, much cheaper than the thousands for PET scans or CSF tests.

And it could help catch the disease early. Amyloid plaques start to build up and release peptides into the bloodstream long before symptoms such as memory loss appear. Irreversible and substantial damage to the brain has usually already occurred by the time people with signs of the disease, like O'Bryant's grandmother, finally see a doctor and begin taking medicines, such as memantine or cholinesterase inhibitors, that can alleviate cognitive symptoms like memory loss, confusion, and other cognitive symptoms. Having a blood test would be "valuable to help these patients early on," says Richard Batrla-Utermann, medical director at Roche Diagnostics.

Ultimately, researchers hope that having a blood test will make it easier to develop better AD treatments. Lacking a way to cure or reverse AD has slowed the drive to develop such a test. But a blood test is crucial to a cure, since it will help identify participants for clinical trials to test candidate drugs. "Without a doubt, having one will help the other," Bateman says.

Jyoti Madhusoodanan is a freelance writer. A version of this story first appeared in *ACS Central Science*: cenm.ag/alzheimersbloodtest

CREDIT: QUANTERIX

It is now time to move on to the next LC - IR separation spectrum,

We see that tube 1-8 on Dec 22 2016 shown some additional activity.

We now have peaks @

same 1106 strong, (range ~ 1200-1000)
same 612 medium (narrow)

~same ~1650 weak, wider

same ~1348 weak, wide

836 weak

685 weak

No CH just present available. Once again suggestive of a sulphate compound.

Also notice our pH is now ~ 9.5

We see also that we have essentially two additional weak peaks @ 836 & 685 peaks

Using SDBS shows that we must focus on the two strongest peaks once again, to keep the sulphate ions in view.

Parameters are 1106 cm^{-1}, 612 cm^{-1}, +/- 10 cm^{-1}, < 30% Transmittance
We must only add 1 additional peak @ a time to investigate influences, suggest ≤ 50% transmittance for first run:

Add 1650 cm^{-1}: Still holds to sulfate Compound.
Guanadene sulfate

$HN=C(NH_2)-NH_2 \cdot 1/2\,H_2SO_4 \cdot 1/4\,H_2O$

Now substitute 1348 cm^{-1}:
Best match: Methyl hydrazine sulfate

Substitute 836 cm^{-1}; No pattern apparent.

Substitute 605 cm^{-1}: No apparent pattern.

Looking in general, sulfuric acid Continues to look like the most Closest match.
Recall that our pH has been altered in both cases to ~7.4 & ~9.5.

Now let's look @ Parker for any additional information.

Start w Parker, Inorganic Analysis, p 402.
Also pay attention to pH

		pH	
*	1106	~7.5	SO_4^{2-}
	612	~7.5	Nothing
	1650	~7.5	Not available (Parker has nitrate 1600-1650)
*	1348	~7.5	NO_3^- Also important. Nitrates!
*	836	~9.5	HPO_4^{2-}
	605	~9.5	Nothing

Therefore we pick up two matches.

The conclusion of LC-IR analyses on with Tubes 1-5 & 1-6 on Dec 22 2018 is that we have both sulfate and phosphate ions in the solution.

There is strong reasonable w/ knowledge of the culture methods and also the detection of sulfuric acid.

Phosphate ions $H_2PO_4^{2-}$ are now added to the inventory list of Feb 05 2019

Nitrate ions (NO_3^-) have also been added to the inventory list. There is also completely consistent w/ previous bacterial metabolic research (ie, bacterial infections).

Primarily, you are detecting polyatomic ions @ this point.

Nitrates, sulfates, phosphates

Sulfuric acid Phosphoric Acid Most likely See June 15 2019

Protein

Thiocyanates

~~Iron~~ Iron Fe^{+3} (Coordination complex).

Very strong evidence of alcohol (IR spectrum, NIR spectrum)

Next we move on to the IR spectrum of Tube 2-2 on Dec 22 2018.

Parker P 104

2969 cm^{-1}	strong	~2960 C-methyl CH_3
2885 cm^{-1}		2875 C-methyl CH_3
~1105 cm^{-1}	→ SO_4^{2-}	

1380	med	Methyl CH_3 (1385-1375)
1630	med	1625 Ph conjugated C=C, Nitrate (1600-1650)

938	weak	N-O (930-960)
682		690 C-H (RHC=CHR') cis

pH was 12.2 but also was neutralized for use in IR ATR.

What we see here is that a hydrocarbon (CH_3) methyl group has definitely been brought into the picture.

We also see potential replication of the Nitrates, NO bond, or the introduction of a double carbon bond.

Now let's go to Tube 2-3.

Clearly there has been the mis-assignment of the aldehyde functional group - this will be rectified as the work carries forward.

At this point, we are dealing w/ a methyl group, not an aldehyde.

Peaks are:

also 2985 & 2941

2864 strong

(notice mono-substitution ~ 2990 which favors indicate hydrocarbon groups).

same 1100 strong SO_4^{2-}

1594 weak - medium
1448 weak - medium

939 weak

—

Hold on. The aldehyde determination may not be incorrect

Aldehydes indeed have a range of 2705 - 2900

This is very important.

This is getting interesting. Aldehydes are going to deserve special review.
Two sources, both Parker & Pavia state that aldehydes display two peaks.

Parker states two peaks (weak @ that) between 2705 - 2900

Pavia also states two peaks, one near 2850 and the other 2750, and that they are also weak absorptions @ that.

Pavia shows an example, and of the classes from that I am familiar with, they do not especially weak, and they tie in on the right side of CH peaks.

Now, let's back up to the spectrum of Table 2-2, with peaks @ 2969, 2885 & 1380 cm^{-1}.

All of these indicate methyl CH_3.

None of them coincide w/ Pavia's rather specific information as well as example plot.

The theme continues to confirm the assignment of each of these three absorption peaks to their methyl group.

It is of interest that there is no strong absorption near 3000 cm^{-1} on Table 2-2. But methyl group continues to be confirmed.

Then says that @ the point of Tube 2-2 analysis that we have the introduction of a



compound. The compound is independent, it would seem, from the polyatomic ion previously identified.

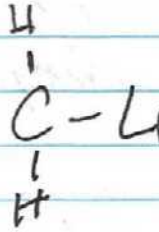
Now, it is OK to proceed once again to tube 2-3

Our absorption peaks, once again, are

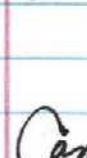
2965 (s)	1100 (s)	1594 (w-m)	934
2941 (w)		1448 (w-m)	
2864 (w)			

↑ let's start this w/ SDBS.

An interesting result here already. The first (and also quite simple) compound that shows up in the SDBS search (+/- 10 cm^{-1}, ≤ 80% T) is Methyllithium.



Basically we have



Cannot get much simpler than this.

What is also interesting in the spectra is the complexity of the spectrum in the fingerprint region.

Recall that the methyl group, alone, however, shows up @ ~1380 cm^{-1} (1375-1385)

The explains some activity but interesting that there is also a major peak around 1100 cm^{-1}

Li is a group I element (e.g. Na, K, also)
Does the methyl group combine w/ Na or K?
No, not at least as SDBS database is concerned.
I wonder why it joins w/ lithium?

We might be able to learn something about R?
Our target peaks are 1594, 1448 & 934

1594: Parker Candidates are:
~1600 C=C (vs) - not the case?
* 1560-1620 (ms) NH_2^+
1585 (m) NH_3^+ amine salt
1585-1625 (m) C=C aromatic
1550-1650 (w) N-H secondary amine
1580-1650 (m-s) N-H primary amine

The width of our band is wide, ~1500-1700 for total range.

It seems to me that our most likely candidate is NH_2^+ or NH^{3+}, i.e. an amine ion, amine salt categories. NH_2^+ may even be preferred

The charge back into play, in a rather forward way, the prospect of the ammonium sulfate ideal

Ammonium sulfate is $(NH_4)_2SO_4$

this means NH_4 has a charge of +1.

Is there a NH_2SO_4? Apparently not.

Our strongest candidate is NH_2^+

NH_2^+ is called a "nitrenium ion"

It is a reactive intermediate

* "Aryl nitrenium ions are currently investigated because of their involvement in certain DNA damaging processes"

They are intermediates in organic reactions

* Nitrenium ions can induce DNA mutations

—

Our next strongest candidate is NH_3^{3+}

Cations have a positive charge
(Remember, electrons enter the cathode)
Therefore cathodes must have a positive charge
in order to attract those ~~e~~ electrons)

So NH_3 is ammonia.
NH_4^+ is a cation called ammonium.

NH_3^+ is called an "amine salt" by Parker.
Yahoo Answers states that NH_3^+ ions do not exist.
Let's work w/ the amine salt approach from Parker.

"Amine salts are salts similar to ammonium salts in which the hydrogen atoms attached to the nitrogen are replaced by one or more organic groups".

"Amines readily form salts w/ acids"

"Salts formed by amines are crystalline substances that are readily soluble in water."

If alkali (eg KOH such as I did to neutralize the solution) is added to such solutions of salts the free amine is liberated.

Tube 23 has a pH of 12.9

Well, this seems to fit well. The benzoic back ammonium sulfate back into the picture.

Back to SDBS.

Before this, however, definitions:

An amine salt is $RNH_2 + HCl^-$

An ammonium salt is ~~RNH_2~~ $R_4N + A^-$

where R is hydrogen, alkyl or aryl groups and A is an anion.

Ammonium sulfate is an ammonium salt and an inorganic sulfate salt.

Example of amine salt:

$$(CH_3)_2NH + HCl \rightarrow [(CH_3)_2NH_2]^+ Cl^-$$

Dimethyl Amine Hydrochloric Acid → dimethyl ammonium chloride

Ammonium sulfate has 4 strong peaks:

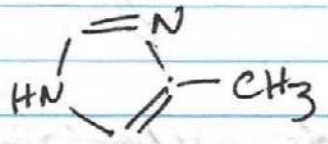
~3120
~1400
~1105
~610

This really is not our match because of the methyl absorption domination and our absorption @ ~1594

We have

2985 1100 1594 934
2941 1448
2864

Methylimidazole has my interest. $C_4H_6N_2$



There is one best SDBS match using
2985, 1100, 1594 cm^{-1} (+/- 10 cm^{-1}, ≤ 30%)
and especially so by forcing a methyl into
the name of the compound.

What these are azoles?

Next, if we include 934 cm^{-1} in the search, we

2985, 1100, 1594, 934 (± 10 cm^{-1}, ≤ 50% T)
and force methyl into the compound name
We are led to the same compound
4- methylimidazole

This seems to be the best type of lead at this point.

Alcohols & azoles are to be added to the inventory.
of candidates

Azoles are used medicinally as anti fungal agents.

Notice the appearance of the C=C bond appearing
with 1594 cm^{-1} absorption along w/ NH stretch.
This could be supportive to the methylimidazole
case as well.

February 10 2019

The Compound is a new one on the block for us.
Methyl imidazole

The well now be on the lookout for investigation.

Before we go further, let's establish how strong the case is for the alcohol:

It should be quite strong. We will see the further as we approach the final protein spectrum.

1. IR absorption shows the broad peak (strong) characteristic of alcohol absorption. Amine absorption will be weaker.

We will see this in more ways than one but table 3-1 & 3-2 on Dec 24 2018 will make the case that alcohol & amine are both present. The broad deep absorption @ 3314 cm^{-1} will be from an alcohol and the amine should be near 3500 — not so clear there yet. But the broad peak @ ~3314 says alcohol.

2. NIR absorption also says alcohol which took place on Feb 02 2019. Strong definite peak absorption localized @ 946 nm. 946 nm, strong, definite ~~Centralized~~ peak definitely says alcohol, and ArOH @ that.

See June 15 2019 Notes on Phosphoric Acid

Intermediate Protein Inventory

Therefore are current inventory of the soluble protein is:

1. Iron, Fe^{+3} state, coordinated complex
2. ~~Sulfuric acid~~ H_2SO_4 Phosphoric Acid
3. Nitrates, sulfates, phosphates
4. Alcohol – ~~AvOH~~ under strong consideration – propanol... etc
5. Thiocyanates ~~Ethyl alcohol is best assessment.~~ ↑ ? ? ↑
6. Protein (ethyl alcohol, best estimate)
7. Methyl group (now a strong candidate)
8. Methylimidazole (~~suggested & under consideration~~)
9. "Polymeric Intermolecular OH bonding" (Notes 02-18-19)

poly vinyl ~~ethyl~~ alcohol

Poly vinyl alcohol (polymeric)

[See Notes 02-14-19 Phenol question remains!!!]

On we go to the next spectrum in the LC-IR series

We have just finished w/ Tube 2-3 of Dec 22 2018.

10. Fermentation Process

Notice that the aldehyde conclusions are not warranted @ this stage. This must be fully considered w/in all discussions that have recently taken place (esp. Vol 23 & 24 of notes),

Now look @ the extended investigation of Tube 2-3 on Dec 23 2018 where we presumed that we had an aldehyde. The tube was heated and a simplified, pronounced IR spectrum was obtained.

First off, a further review of the "aldehyde" situation.

From two sources, we expect two peaks (~2850 & 2750 cm^{-1}) to have an aldehyde.

We do not have this.

Tube 2-3, in the heating experiment, shown peaks @ 2920 cm^{-1} (weak) and 2867 cm^{-1} (strong).

* There is no peak @ ~2750 cm^{-1} and therefore the aldehyde requirement IS NOT MET.

2920 is Classic hydrocarbon absorption.

The 2867 cm^{-1} absorbtion (strong) is the question.

Parker shows ~2875 cm^{-1} as methyl group.
Pavia states that methyl absorption also takes place @ ~1375 cm^{-1}.

Parker confirms the secondary methyl absorption by stating 1375-1385 as the methyl group.

Tube 2-2 definitely shows the presence w/ both absorptions @ 2885 cm^{-1} & 1380 cm^{-1}. Recall that our pH here is ~12.9, highly alkaline.

Now suspect Change for Tube 2-3. We see it is still alkaline @ pH ~ 12.9.

We do have strong absorption @ ~2867 cm^{-1}. There is no visible absorption @ ~2750. There may be absorption @ ~1380 but it is not dominant. The pattern considers to establish the presence of the methyl group and NOT an aldehyde.

Now we look @ the testing of Tube 2-3 from Dec 23, 2018.
We see a couple of interesting changes.
For one:
We have an extremely strong peak that appears @ 1437 cm^{-1}.

From Parker,

This is smack in the middle of the azo range with a functional group of

$-N=N-$

Now, isn't that interesting in light of our notes regarding the suspected candidacy of methyl imidazole.

We obviously need to look @ the spectrum more closely.
Our peaks are now:

2929 (w)
2867 (s) CH_3

1437 (s) Azo $-N=N-$
(1079) (s) $H_2SO_3^-$, $H_2PO_4^-$, $H_2PO_4^{2-}$ Inorganic
most likely
1597 (m) C=C @ 1600 via Parker (see notes Feb 09 2019)
1662 (m) Parker 1662-1652 C=C
~1670 C=N

We continue to stay in the same territory
methyl, C=N, C=C, $-N=N-$
methyl imidazole remains under consideration.

SDBS next.

When we run the SOBS search, we once again have 4-methylimidazole as a front runner candidate.

Parameters of search are:

2867	T = 8	} diagnostic absorption peak @ each of these locations.
1437	T = ~20	
1591	T = ~40	

(+/- 10 cm^{-1}, ≤ 30% T)

The compound now becomes a leading candidate and is suitable to add to the inventory.

Methylimidazole is an aromatic heterocyclic compound $CH_3C_3H_3N_2$.

* Colorless. It is a fundamental nitrogen heterocycle and as such mimics for various nucleoside bases as well as histidine and histamine.

A precursor for the synthesis of polyamides.

* These polymers can selectively bind specific sequences of double stranded DNA.

Feb 12 2019

Some personal health comments to keep all in perspective:

1. Last May (2018), immediately prior to the Tucson Conference I have good reason to believe that directed efforts to "terminate my existence" may well have taken place. I experienced very unusual effects and events upon my heart during these events. They were severe and I regard myself as fortunate to have survived. My best assessment is that I experienced a case of unstable angina during that week period. The probability of a directed effort should not be discarded. Other individuals participating or involved w/ the conference also have reported similar "targeted" experiences.

2. Last summer, during the seasonal migration to MT and the northwest, I lost the use of my arm (right) almost entirely for a period of 3-6 months. It appears that, as a phenomenum, a nerve was severely pinched. The situation is complicated by the fact that I have had resident chronic pain in my right shoulder. ~~Had a~~ that also seems to be tied into chronic low level congestion in my upper right lung-shoulder area.

3. In addition, I have experienced chronic low level fluid ear wax drainage from my right ear over the last couple of years. At times this results in temporary deafness until I can get the wax cleared. Two years ago the blockage became severe, affecting both ears, and almost total deafness ensued for approximately three weeks.

4. From the recent ultrasound examinations (optional and for informational purposes) we know that I have:

1. A 2.5cm nodule - cyst in my right thyroid

2. Gall stones

3. 10% blockage in the left femoral artery.

5. It is quite possible that the lung congestion, the right shoulder pain, the low level ear wax drainage and the thyroid condition may all be related to one another. The existence of a biofilm - filament network formation in the upper right quadrant of the body must be seriously considered.

6. During the months of Nov 2018 - Dec 2018 the widespread "flu" condition took hold.

It is not a "flu".

The research paper entitled "Another ~~Marker~~ Marker for Examination" explains & shows the actual cause of this "condition". It is a biofilm - filament network within the lungs. The evidence has now been captured clearly and equates directly to the culture growth (CDB).

The episode was severe, as it has been many times ~~over~~ over the years. What is different is that I am now more successful in accelerating the recovery period. Two months were required to improve affairs; in the past the conditions have persisted up to years, at least w/ the chronic cough.

The three primary methods of assistance are -

1. Colloidal silver - internal
2. Methyl salicylate - ~15-20% in balm over the affected areas (right lung, shoulder, etc) (serendipitous discovery)
3. Ultrasound

7. Over the past week, I have sustained a rather severe gall bladder attack. It reached the point when the urine turned brown in color, from the extent of bilirubin in the urine.

I have passed two gallstones, the second was larger than the first. I will estimate the size of the first as ~~3mm~~ 3-4 mm, and the second as 5-6 mm. The estimate is based upon the rate of passage along with study by the xrays of the cystic duct and the common duct.

Digestion became literally almost nil during the event and I regard the symptoms as severe, with full blockage a concern.

It is to be stated that the use of ultrasound WAS OF GREAT ASSIST in helping to move these gallstones through the bile ducts. The progress of movement of both stones could also be readily monitored w/ the use of a therapeutic ultrasound device (~~not for~~ no imaging capability in this case).

In general, over the past year, it has been found that ultrasound has many beneficial capabilities, both therapeutically and for imaging.

It is thought that this technology should be explored fully for its potential advantages, including those that appear to result directly or indirectly from the CDB microbial effects upon the body and human health.

The serendipitous benefits of methyl salicylate (even as they are applied externally) should also be thoroughly researched, including that of potential impact upon the microbial growth.

—

Now that we have the introduction of a possible another potentially significant compound (methylimidazole or similar or related compound), let's move on to the next spectrum. This will follow the heated methyl separation. This was Tube 2-3 from Dec 23 2018.

Feb 14 2019 - Valentine's Day
In the desert, about 30 miles N. of Tucson.

Now I look @ Tube 2-5 of Dec 22 2018.
This was the end of an LC run. We note that the eluate showed a slight purplish tint and that the pH was 12.1. We used HCl to produce the eluate.

We can see from the IR spectrum that the methyl group is no longer showing up. We have peaks @:

1062 (strong)
1597 (weak)

1597 gives the C=C @ 1600 via Parker, previously discussed.

1062 cm^{-1} appears to be a hint of a shift from the previous recent IR analyses.

Looking @ inorganics sent, HSO_4^- appears to be the closest match from Parker inorganic Chart p402. He has HSO_4^- (vs SO_4^{2-}) @ ~ 1058 cm^{-1}.

There is one peculiarity. The peak is broad and strong.

The organic candidates do not seem especially strong @ the point. (Parker)

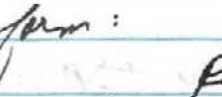
1030 - 1090 P-O-C Phosphate ester, very strong
1020 - 1090 Si-O no source of silicon known @ the point.

Both silicon and phosphorus are "metalloids". (semi metals)
An alternate periodic table does not place phosphorus in the semi-metal category - it is classified as a non-metal there.

Esters require the presence of a carbonyl group (1600 - 1820 cm^{-1}) and we do not have that, therefore the weight of evidence continues to support the inorganic ion HSO_4^-, a variant of the HSO_4^{2-} ion, which seems to be reasonable.

No significant additional information appears to be forthcoming from this rather recent spectrum.

Incidentally, esters are of form:



and the carbonyl group would show up @ ~ 1735 cm^{-1}. No go.

Next we have a repeat / concentration LC run. Our tube series is 3-1 to 3-7 on Dec 24, 2019. We only have spectra for that tube that produced a marked result / signal.

Our notes of Dec 24 2019 that accompany the LC runs are significant to keep in mind here. Notice comments related to potential residuals in the column, immediate elution from the column, film presence upon evaporation on the ATR plate, etc.

The first thing we notice is that Tube 3-1 presents a complex spectrum relative to former spectra, and therefore is likely to be showing a combination of compounds. This is where the sequential analysis of LC-IR runs and spectra are valuable.

Let's look @ our peak set:

~3275 Very strong broad absorption
1023 Very strong broad absorption.

from Parker:

3225-3400 (Strong & broad) Intermolecular bonded OH Polymeric

This is our best match for this broad strong peak we have ever identified thus far. We will get to superimposed variations on this absorption @ a later time.

It is very interesting to see that Parker is making an assignment to a polymeric OH - this shows we need to examine the variation within the alcohols.

Now notice that Pavia has conventional OH alcohol/phenol absorption between 3600 - 3300 cm^{-1}. We are slightly outside of the range which suggests that we are not in that conventional OH category.

From Pavia, furthermore, if hydrogen bonding is present (our conclusion is definitely yes) w/ a conventional alcohol/phenol the range is 3200 - 3500 w/ broad, strong absorption. The center of the absorption is ~ 3350 cm^{-1}. We are, however @ ~ 3275.

Parker actually only gives one reference however, to STRONG, BROAD OH absorption (3400 - 3225, center @ ~ 3312)

NOT TRUE clc 02-18-19

which happens to coincide with the center average of our center of absorption and the center of the range given by Pavia. How is that you introducing an element of confusion?

Basically, it is unknown as to the exact cause of the term "polymeric" by Parker, at this point. Let us look further into this.

It would appear to me from the example alcohol plots given by Davia, it seems that the aliphatic alcohol has centered broad absorption near ~ 3350 but that the aromatic alcohol also has centered absorption near ~ 3330 cm^{-1}.

We are to the right/slightly of both of these (~3225) and it raises the question of whether "polymeric" structure induces that shift.

OH bonding: 3 Types:

Let's look into Polymeric OH absorption.

(1) A primary difference appear to be that between ~~[illegible]~~ (3590 - 3425 cm^{-1}, center ≅ 3501 cm^{-1}) intra molecular sharp absorption of bonded OH

~~and Polymeric (3400-3225, center ≅ 3312 cm^{-1})~~

(2) or

Dimeric inter molecular bonded OH at

3450-3550, center ≅ 3500 cm^{-1} Sharp absorption

(3) or

Polymeric inter molecular bonded OH @ (3225 - 3400 cm^{-1}, center ≅ 3312 cm^{-1})

Clearly, C ~3275 cm^{-1} we are closest to this last category, as are both example (both aliphatic and aromatic) of alcohols given by Pavia VIR spectra.

Pavia theoretical center point absorption : 3312 cm^{-1}
Tube 3-1 approximate center absorption — $\bar{x}$ = 3308 cm^{-1} [3275 cm^{-1} ~3340 cm^{-1}
Pavia alcohol example spectra

Therefore we can splot no further here. My absorption and Pavia example absorption (both aliphatic and aromatic) are all in the same general league.

Polymeric inter molecular OH absorption appears to be the norm.

—

Therefore, we know that Tube 3-1 displays:

"Polymeric, intermolecular OH bonding"

i.e., classic alcohol structure.

We therefore confirm further our conclusion of an alcohol present in the soluble protein form, further confirmed w/ NIR.

Recall that NIR do suggests an aromatic OH and the remainder subject to further examination.

What remains in Confusion however, is the memory of a failed test for phenol existence in the past.

Question: 1. Are all aromatic alcohols phenols?

2. Do we have a failure for phenol testing?

Now, we move on to 1023 cm^{-1}.

From Parker, Inorganic chart,

PO_4^{3-} is a perfect candidate here @ ($\sim$1020 cm^{-1})

One question here, however, is that Parker testing assumes a pH > 14, which is definitely not what we have. Our pH $\cong$ 7.6,

There is no other inorganic competition here.

Notice that Parker gives a range of 1000–1200 w/ strong absorption for alcohol alcohols. C–OH bond.

Now that is interesting, isn't it?
The center of the range is $\sim$1100 so we are dependent off center here.

Now here are a couple of observations:

1. The range of correlation observed absorption via Parker is 1000-1200. Even though the center of the range is 1100, the example alcohol given by Pavia (aliphatic form) shows a center of absorption @ ~ 1020 cm^{-1}.

And this is right where we are @ 1023 cm^{-1}.

Therefore our absorptions @ 3275 cm^{-1} & 1023 cm^{-1} are in perfect keeping w/ the aliphatic alcohol example given by Pavia.

We most definitely have an alcohol well established here.

2. We also note in the aromatic alcohol example given by Pavia that the absorption is @ ~ 1040 cm^{-1} and is not nearly as strong. The same the case in the ~~aliphatic~~ alcohol. Only the NIR absorption is remaining as suggestive of the aromatic alcohol. The phenol test will also be repeated to try and settle the difference on this.

3. I notice also that the aliphatic alcohol example by Pavia does show some irregularity of absorption w/in the 3200-3400 range.

Use is important because the means you must be cautious of attaching such irregularity to that of amine absorption, as you may be inclined.

In the example case, the irregularity is focused @ 3300 cm^{-1} and Pavia assigns it to an alkene bond. The alcohol example is $HC \equiv C-CH_2-OH$ (propargyl alcohol)

Each of these subtleties in observation are potentially important. We shall continue to study the absorption patterns in detail but let us just limit that methyl alcohol may well be on the horizon here.

It is now time to proceed to the next sequence of absorption.

The next absorption of interest are the peaks @:

2929 cm^{-1}
& 2886 cm^{-1}.

Via the notes of Feb 04 2019, these are a resurfacing of old friends and hydrocarbons

From Parker,

~ 2925 cm^{-1} is $>CH_2$, methylene, or $Ar-CH_3$
~ 2875 cm^{-1} is the methyl group.

additionally:
~ 2960 is a methyl group
2900 - 2880 is the C-H, methine group.

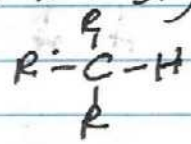
Via Parker also, the aldehyde double peak group falls w/in 2900 - 2705.

We have 2929 cm^{-1} & 2886 cm^{-1}.

Additional ~~above~~ source information:

IR Spec gives 2925 cm^{-1} as methylene $=CH_2$
2870 cm^{-1} as the methyl group $-CH_3$
2890 cm^{-1} as the methine group $=CH-$

Methine is a trivalent functional group
It is also listed as C-H
(ie, methane minus three hydrogen.
The means



Spectramate gives 2925 @ $R-CH_2$
2886 @ $R-CH_2$

SDBS search using 2929, 2886 cm^{-1}
(+/- 5 cm^{-1}, ~~I ≤ 20~~). T 20, 80)

gives

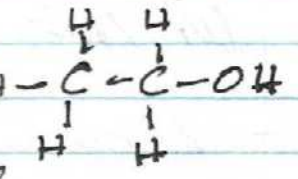
~~$HC\equiv C-CH_2-NH_2$~~ $HC\equiv C-CH_2-R$

CH_3-CH_2-R

$H_3C-CH=\underset{\underset{CH_3}{|}}{C}-CH_3$

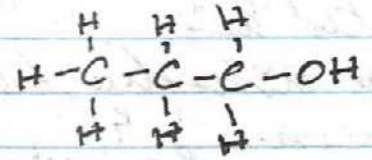
(Let us +/- 5 cm^{-1}, T ≤ 50):

CH_3-CH_2-R

C_2H_6O - ethyl alcohol 

C_3H_8O $CH_3-\underset{\underset{OH}{|}}{CH}-CH_3$ Propanol

$CH_3-CH_2-O-CH=CH_2$

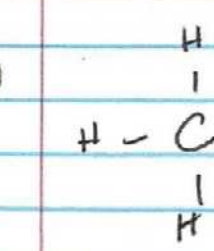
Propanol is 

We can see some interesting results from the SDBS search under fairly tight parameters ($\pm 5 cm^{-1}$, T≤5%)

What we see is that the alcohols are indeed prominent. Also the methyl & methylene groups appear to be almost assumed functional groups.

Our best analysis here would be the simplest alcohol that allows for a methyl group, a methylene group, and OH.

This is:



This is, in fact, ethyl alcohol C_2H_6O. (Methanol would not have the CH_2 group.

This is our best identification of the alcohol that is involved and appears to be a very reasonable assessment. The inventory will be correspondingly modified (Feb 10 2019 notes)

* "Alcohol (ie, ethanol) promotes the accumulation of fat in the liver. It also progressively alters the mitochondria. The mitochondrial damage perpetuates fatty acid accumulation".

PubMed, Baraona, 1979

If the compound were bumped up by one additional carbon to become propanol instead of ethanol,

* Quite flexible - completely consistent w/ IR.

Then we have a compound that is 2-4 times more toxic than ethanol

and it becomes a Central Nervous System depressant.

* DNA Testing: First Attempts

Feb 10 2010.

In the interim, a personal visit to an accredited laboratory for DNA analysis ~~has been made~~ took place. The CSB DNA sample was hand delivered to the lab in an effort to seek a preliminary analysis.

Analysis to the level of domain knowledge only was established as a condition of testing, any identification beyond that was negotiable in terms by cost.

The is a viable sample and the notes for the process of extraction have been recorded in my volumes. I have carried the sample since NOV 18, 2010 without incident. The sample is with a small amount (~2ml) of denatured alcohol.

During travel from Tucson area to Phoenix the tube did leak about 1/2 of the alcohol, but there was no loss of sample and it remains perfectly intact and viable. There was unfortunately no actual consequence to the viability of the sample.

At the point in time, the response of the lab is that the sample is being rejected for analysis.

The position of the laboratory is that the "purity" of the sample cannot be assessed and therefore productivity from the analysis process is also not assessed.

to I have been given a contact person to discuss requirements for further submission of any samples.

I will let the information set without further comment for now. It is more beneficial to let the situation unfold further before any speculation is introduced into the discussion.

To now, analysis of the CDB DNA sample by an accredited lab is on hold.

For the record, an attempt has been made. I regard the sample as perfectly viable and pure enough to warrant analysis. I make no claim that the sample is "pure DNA", in fact I would never suspect so. I would presume, however, that it is more than adequate to provide for further purification and reproducibility in the lab, i.e. via PCR techniques and with the expertise of the lab available.

I will retrieve the sample, hopefully along with the chain of custody submission.

Let us move on, with the current IR spectrum; our last analysis provides further evidence of an aliphatic alcohol (w/ in the soluble protein.

We are working w/ Table 3-1 and the notes of Feb 14 2019.

Further comments on the DNA submission.
Please Please note that corroborating information for the DNA sample includes:

1. 260/280 Ratio: Purity estimated @ ~ 0%.
2. Microscopy of banana control against CDB DNA.
3. IR spectrum comparison of control DNA against CDB DNA.

All evidence highly supports the claim of an adequate DNA sample.

Then yet, I have not been asked for any such information nor have I been offered the opportunity to present it. I am not yet in any direct contact w/ the actual lab personnel.

Before we proceed w/ additional peak absorptions, I can see that it was improper to take an average of ~3275 cm^{-1} for the alcohol absorption.

Indeed the strongest absorption is @ 3312 cm^{-1}

which we see is also the exact absorption of the center of the range of Parker (3225 - 3400 cm^{-1}) for

Intermolecular bonded OH polymeric

---

so we know that we have the:

There is no ambiguity here and this phrase is now to be added to the inventory comparison list. What we do not yet know is how the compares to that of an "aliphatic alcohol" assessment that I have made.

Let's look into this phrase a little deeper:

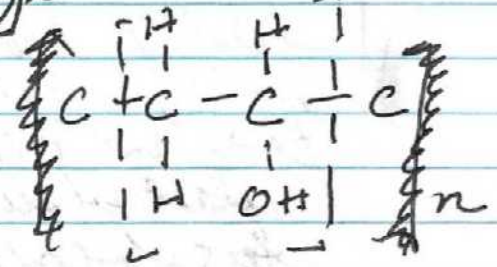
Cyclohexanol  (not a benzene ring)

is one example of a polymeric OH bond, apparently.

* Important observation here:

Poly(Vinyl) alcohol appears to be a primary example. A water soluble synthetic polymer.

Idealized formula of $[CH_2CH(OH)]_n$



White, colorless, & odorless

The is very interesting. Notice that it is a chain.

* No oil is required to produce it. It has excellent film forming properties. Also adhesive.

* Notice our comments on Dec 24 2018 (Item 16.3) & specifically noted that the particular tube (3-1) of the LC-Lens evaporated to "a thin shellac film layer" on the ATR plates, exactly as we have seen formerly w/ the fuel protein solution.

—

We now have very strong evidence that we do indeed have polyvinyl alcohol here.

* It is of interest that there is an aliphatic jump. You could easily have a methyl group from one of the Carbons and the chain could easily carry on.

* It is also of interest to note that it does say that it is produced synthetically.

This is, of course, a very big deal.

I have seen signs of this for many years. Today is the first confirmation @ a higher level of evidence and analysis.

Borax and PVA will make a form of plastic (slime) - this can be the basis for confirmation.

Question - Do any known bacteria produce polyvinyl alcohol, or similar/related compounds?

Biomedical applications are one of the hot topics of research, as anticipated.

Artificial organs, artificial tissue development, attachment to bone, artificial vessels, prosthetics, etc

We are dealing w/ a "bio-compatible" form of plastic here.

" The case for transhumanism ", anyone?

The case has just become much stronger I would say.

Next up is the borax test.

I have never made it to the additional IR peaks of Table 3-1 today.

That is groundbreaking information enough for today.

Best to settle on this for a while and seek alternate test methods for confirmation, eg borax.

Feb 21 2019

The EMSL laboratory in Phoenix has refused to accept the ~~signed~~ sample of DNA.

All communications thru me are indirect via a salesperson on staff. No direct access to the lab personnel that are located in New Jersey. The lab in Phoenix does not have DNA capabilities.

The best word I have at the time is that the NJ main lab wants direct access to the microbe and a culturing process.

They are not going to get this information from me as they are not entitled to it and it is not necessary to perform the services needed.

I have been given a referral to the lab manager and I will plan a discussion w/ that person later. I will not be back at the lab until May 2019.

The lab staff in Phoenix was not able to answer my questions adequately; the sample provided was most certainly viable and adequate control information to establish the presence of DNA was made known to them. They had no interest in this substantiating information @ this time.

Email of record also to be made available.

DNA Testing Chain of Custody

EMSL Order Number (*Lab Use Only*):

EMSL Analytical, Inc.
200 Route 130 North

Chain of DNA Custody Information
Feb 2019

EMSL ANALYTICAL, INC.
LABORATORY · PRODUCTS · TRAINING

DNA Testing Chain of Custody

EMSL Order Number (*Lab Use Only*):

EMSL ANALYTICAL, INC.
200 ROUTE 130 NORTH
CINNAMINSON, NJ 08077
PHONE: (800) 220-3675
FAX: (856) 786-0262

Company Name: Carnicom Institute	**EMSL-Bill to:** ☐ Same ☐ Different If Bill to is Different please note in Comments**
Street: 915 200E - PO Box 1224	*Third Party Billing requires written authorization from third party*
City: Monticello **State/Province:** UT	**Zip/Postal Code:** 84535 **Country:**
Report To (Name): Clifford E Carnicom	**Fax #:**
Telephone #: 520.330.1421	**E-mail Address:** CEC102@gmx.com
Project Name/ Number: CI - DNA Analysis	**Project Zip Code:** 84535
Please Provide Results: ☐ Fax ☑ E-mail **PO#**	**U.S. State Samples Taken:** UT

Turnaround Time (TAT) Options - Please Check

☐ 3 Hour ☐ 6 Hour ☐ 1 Day ☐ 2 Day ☐ 3 Day ☐ 4 Day ☐ 1 Week ☑ 2 Week

Fungi	Bacteria	Insects
☐ ERMI Panel (M180) *Dust Only*	☐ Human *Bacteroides* (M199)	☐ Bed Bug (*Cimex lectularius*) (M146)
☐ EPA 36 Panel (M233) *Air, Swab*	☐ Total *Bacteroides* (M095)	☐ Tick - *Anaplasma phagocytophilum* Anaplasmosis (M261)
☐ Water Damage 20 Panel (M181)	☐ *E. coli* O157:H7 (M140)	☐ Tick - *Babesia microti* Babesiosis (M260)
☐ *Aspergillus* 15 Panel (M186)	☐ *E. coli* (M200)	☐ Tick - *Borrelia burgdorferi* Lyme disease (M196)
☐ *Aspergillus* 6 Panel (M188)	☐ Total *Enterococcus* (M096)	**Other**
☐ *Penicillium* 13 Panel (M189)	☐ *Helicobacter pylori* (M207)	☐ *Acanthamoeba* spp. (M147)
☐ Customized Fungi Panel (M100)	☐ *Legionella pneumophila* (M103)	☐ *Cryptosporidium* spp. (M237)
☐ *Penicillium* Mycotoxin 9 Panel (M190)	☐ *Legionella* 4 species-EPA (M162)	☐ *Giardia* spp. (M149)
Birds, Animal Droppings	☐ *Legionella* spp. and *L. pneumophila* serogroup 1 (M164)	☐ *Naegleria fowleri* (M197)
☐ *Chlamydophila psittaci* (M234)	☐ Whole Genome Sequencing and Bioinformatic Analysis (M285)	☐ Food Authentication (F130)
☐ *Cryptococcus neoformans* (M143)	☐ *Leptospira* spp. (M262)	☐ GMO Analysis (F131)
☐ *Histoplasma capsulatum* (M208)	☐ *Mycobacterium avium* (M144)	☐ DNA Barcode Analysis (M195)
☐ Raccoon Roundworm (M236)	☐ *Mycobacterium tuberculosis* (M159)	☐ DNA Sequencing Fungi/Bacteria Isolates (M192)
☐ Rodent (Mouse, Rat) Dropping (M271)	☐ *Salmonella* spp. (M141)	☑ Special Request:
	☐ *Shigella* spp. (F122)	

Sample #	Sample Location	Sample Type	Test Code	Volume/Area	Date/Time Collected
CI-01	Monticello, UT	DNA			11/18/18

Client Sample # (s):

Total # of Samples: 1

Relinquished (Client): C E Carnicom Date: Time:

Received (Lab): Date: Time:

Comments/Special Instructions:
unable to process relinquish to client

EMSL Analytical, Inc.'s Laboratory Terms and Conditions are incorporated into this chain of custody by reference in their entirety. Submission of samples to EMSL Analytical, Inc. constitutes acceptance and acknowledgment of all terms and conditions by Customer.

The initial Borax test has been performed on the soluble protein today.

Here is what we learn:

1. There is no direct, obvious visible polymer formation w/ the combination of dilute protein and Borax.

2. We will let it set for a couple of days and see if any congealing takes place.

3. What is noticed, however, is that the exterior of the test tube becomes sticky upon evaporation. Further testing reveals that this takes place from the protein alone and it does not require Borax to produce this result.

4. What we see, therefore, is that the protein, upon sufficient evaporation DOES have additional qualities to it. This is one of the attributes of polyvinyl alcohol as mentioned on Feb 10 2019.

5. Everything says that we do indeed have a Polyvinyl alcohol compound here. It may well not be polyvinyl alcohol in its most basic state, which only contains the methylene group (CH_2) and no methyl group (CH_3).

Soluble Proteins - Inventory

* The inventory on the protein (soluble) is now:

1. Iron, Fe^{+3} state, coordenated complex
2. Sulfuric acid, H_2SO_4
3. Nitrates
4. Sulfates
5. Phosphates
6. Polyvinyl alcohol - it is also a synthetic polymer (a related compounds)
7. Thiocyanates
8. Protein - Iron-Sulfur Protein (Ferridoxin) (Mar 12 2019)
9. Methyl imid azole (DNA chain formation)
10. Fermentation process is coming... (Mar 05 2019)
* 11. Thioamide - serious thyroid disruption
- Lupus like (Fatigue, joint pain
It now repeatedly (03/10/19) - hypothyroidism
Repeats again: Mar 12 2019. "chronic autoimmune disorder"
12. Olefin (polypropylene) - a synthetic fiber
is now on the list! (Mar 11 2019 notes)
or related compounds

Under consideration of the Borax test, we must easily allow for the fact that the polyvinyl alcohol compound under consideration is simply not in the most basic form.

The IR data, from the beginning, continues to show the strong presence of a methyl group.

Revise Inventory

* The says to me that our strongest candidate is a polyvinyl alcohol or related compound w/ a methyl group attached.

It also brings up the discussion submitted some time ago (years) that the protein solution has potential economic benefits as both an adhesive and a lubricant.

* We know that the viscosity of the protein can be carefully controlled via evaporation. The inevitable the very real prospect of a water soluble lubricant existing there

* We also observe that adhesive properties exist as well

These potential economic benefits have been cited before in earlier notes.

These include: (potential applications)

1. Lubricant (water soluble)
2. adhesive (water soluble)
3. Energy (Redox) production.
4. Waste breakdown
5. Biodegradable plastic formation
6. Medical tissue / organ development

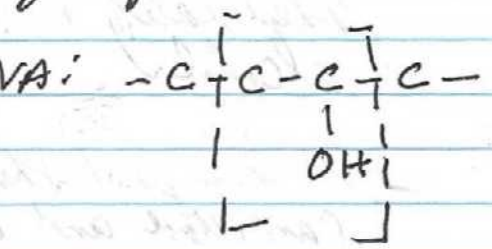
I do not get to see the explored a materials life in my lifetime, however, these opportunities do exist.

Centering w/ R:

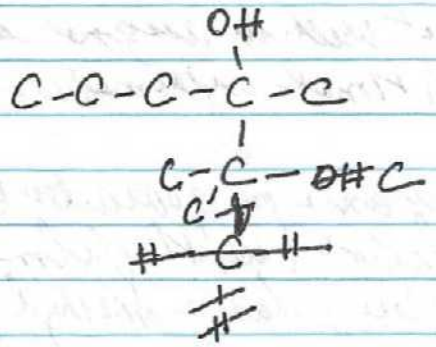
Polyvinyl acetate has a methyl group attached along w/ a polymer chain

however,
it has a Carbonyl group (which we do not show) and it does not have an OH group

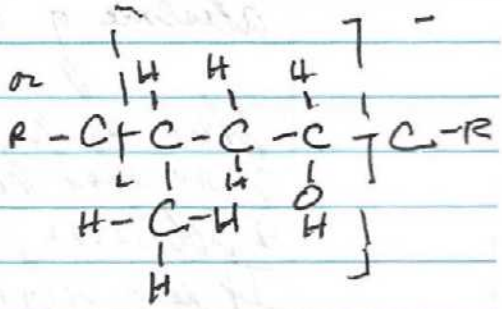
so it does not qualify. PVA:



Consider



or



Let's consider the methyl question.

There are definitely alcohols with a methyl group attached. Think methanol to start. & also we methyl pentanol, there should be methyl propanol, etc!

But @ this point, there is no reason to think that ^ or know these alcohol types are forming polymers.

Recall that the methyl group has been shown up in the progressive series of IR spectra so there is no assurance whatsoever that the methyl group is attached to the alcohol.

What we do know is that the alcohol under review definitely possesses

1. Film attributes
2. Adhesive properties

These satisfy exactly the properties of polyvinyl alcohol

At this point this remains our strongest candidate and it will be assigned as the absence of additional information.

There is no assurance or guarantee @ this point that this alcohol (with film, viscous & adhesive properties) has a methyl group attached. It is a polymer.

Continuing w/ tube 3-1.

We have handled the peaks @ 3312 & 1023.
We assign these to the polymeric alcohol PVA.

We also know that we have a methyl group in the spectrum @ 2929 & 2886.

Our remaining additional peaks are @ 1642 & 1342.

These peaks did show up earlier, for example, see Tube 1-8 that did not even have any hydrocarbons.

This strongly suggests the inorganics again.
1342 indicates the nitrates again (Parker NO_3^-)

Parker also has nitrates @ 1600 - 1650 NO_2
and amides @ 1620 - 1650 NH

We will hold w/ the inorganics @ this point since these are consistent w/ previous findings.

In summary:
1. Polyvinyl alcohol
2. Methyl group
3. Nitrogen inorganic compounds.

Tube 3-4

Now we move on to Tube 3-4 analysis.
pH here is ~7.6 and ORP is −185

This LC elution shows some important differences.
Notice we seem to have lost the methyl group,
and even the deep OH absorption seems to have
left.

We now have especially strong peaks @ 1568 cm^{-1} & strong
1398 cm^{-1} strong
1114 cm^{-1} moderate

Consider inorganics first:

Parker shows a Carbonate @ ~1400, CO_3^{-2}, @ pH >12
Parker inorganics Clear @ ~1560 cm^{-1} and
no candidates there.
1114 Closest match is NH_3 (aqueous) @ ~1107
but pH is also better @ @ pH 11.
Recall we have a pH of 7.6.

Now for Parker organics:

1568
- s 1570 - 1515 NH secondary amide
- vs 1610 - 1540 C-O Carboxylate $-COO^-$

1398
- m 1400 - 1418 C-N primary amide
- s 1420 - 1330 $ROSO_2R'$ sulfonic ester
- s 1440 - 1350 S=O $(RO)_2SO_2$ sulfuric ester

	s	~1120	C=S	-NH-(C=S)- thioamide
1114	s	1100-1150	C-O	benz benzoic ester, phthalic ester
	s	1150-1070	C-O-C	aliphatic ether
	s	1150-1100	S=O	R_2SO_2 sulfone

Mar
Dec 05 2019

Continue w/ IR analysis: Tube 3-4

We have 3 peaks under examination.

I would like to use the process of elimination first. We notice the prospect of an ester for the 1398 & the 1114 cm^{-1} peaks.

However

Via Pavia, we do not even get to an ester unless we have the carbonyl group (1600 - 1820 cm^{-1})

We do not have it. This eliminates the ester. If the carbonyl does not exist, Pavia leads to remaining prospects, eg alcohols, amines, ethers, double bonds, aromatics, nitro groups, etc.

Returning to our Parke Candidates, our list is now reduced to:

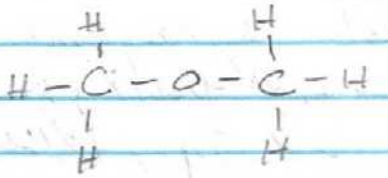
156B:

1568:	S	1590 - 1515	NH secondary amide
	VS	1610 - 1540	C-O carboxylate $-COO^-$
1398:		1400 - 1418	C-N primary amide
1114		~1120	C=S $-NH-(C=S)$ thioamide
		1150 - 1070	C-O-C aliphatic ether
		1150 - 1100	S=O R_2SO_2 sulfone

We cannot help but notice the presence of the amide group common to all peaks. We also notice the absorption above 3000 is also rather weak, and we know now that this is characteristic of the NH bond, especially in comparison to the OH bond.

This now gives us four common data points. peaks.

The simplest ether is Dimethyl ether



C_2H_6O

The require the methyl or CH groups in general and we appear to have neither.

However, notice from Pavia that the carbonyl group is required for the amine. (1600 - 1820)

This seriously raises the question of the thioamide, since it does not require the presence of the carbonyl group.

The general structure of the thioamide is:

$R-CS-NR'R''$

Thioamides act principally by blocking the synthesis of T4.

They treat hyper thyroidism.

This would mean that they are likely to induce hypothyroidism, exactly as would be anticipated.

This is quite profound and it explains the lack of the carbonyl group, a very important part of the picture that has emerged here.

"Thioamides inhibit the enzyme thyroid peroxidase in the thyroid, reducing the synthesis of T_3 & T_4, thereby blocking uptake of iodotyrosines from the colloid.

Hormone depletion is caused by reduced synthesis, which is a slow process."

"The thioamides are associated w/ some side effects, the most serious of which have been a lupus-like syndrome involving the lungs & liver, neutropenia, and elevated transaminase levels".

"Thioamides have immunosuppresive properties".

This is already enough to keep us busy, it would seem.

It certainly is the picture, doesn't it?

				We have
vs	Carboxylates absorb @	1610 - 1540	$\bar{x} = 1575$	1568
		1460 - 1400	$\bar{x} = 1430$	1398

They do not absorb @ ~1120. Thus our primary indicator for the existence of the thioamide, and the assignment therefore remains justified.

Whether or not there remains the parallel existence of the carboxylate $-COO^-$ is also open for further examination.

The appears to handle Tube 3-4.

Our next tube will be Tube # 4-1. (Dec 24 2018)

Mar 11 2019 Cochise Stronghold

Additional observations of ultrasound use:

I have little doubt that the CDB microbe has the ability to form a network w/in the body and that it has the ability to migrate within the body.

The seems to be a classic case of "quorum sensing" and expressive of an "intelligence" for survival, @ the very least. Of course, it may be "mad".

The attribute was first observed when the dental issue developed that eventually resulted in the loss of all lower teeth. In that case a laser light was observed to cause a phototropic negative reaction and cause a migration that literally encircled the tooth under study.

Here we now know that ultrasound has a very substantial effect upon the network, much more influential that migration alone.

In general, in all trials conducted thus far, ultrasound appears to offer us no net benefit in "disrupting" the level of network organization by the microbe.

The trial with ultrasound yesterday was directed to four different targets:

1. The right shoulder (the longer term resident location of pain and inhibition of shoulder movement.

2. The left shoulder - upper left back region (that appears to be a result of migration from the right shoulder to the left shoulder - back region.

3. A developing location of neuroma type pain (a very deep pain (which appears to exist within bone or deep tissue) on the left hand first knuckle. This has been developing slowly over the last couple of weeks and has begun to interfere with general mobility and use of the left hand.

4. The thyroid (right side) where the 1" cyst has been identified via a professional ultrasound examination. This is, without doubt, an entirely experimental process. There is no direct pain in the thyroid region and the benefit/risk ratio of applying ultrasound to the cyst/thyroid target is completely unknown @ this point. There is the understanding that ultrasound (focused) is used as therapy w/ thyroid cysts in other countries; the U.S. is apparently prohibitive @ this time.

The results of the ultrasound (US) application to each target is as follows.

1. The left hand knuckle was the first trial. The US device has 3 power settings: high, medium and low.

The use of high power US caused immediate sharp pain in the knuckle. The pain is immediately unbearable and it was identical to the type of pain that was initially experienced w/ the use of US on the chronic right shoulder situation.

Medium power setting created the same result.

Low power setting was quite acceptable and was applied for ~ 20 min.

At this stage all indications are favorable and the pain may have already subsided to some degree. On a repeat trial I will work towards increasing the power setting to medium.

All signs (ie, identical pain development symptoms) are that the knuckle location is the result of the microbe network location to a different location to attempt to "set up a new shop"

The progress of the level of knuckle pain and mobility will be monitored. Based upon the results that have occurred with the right shoulder, I anticipate favorable results.

2. The 2nd section w/ US applied was the left upper back (harder to access but manageable) the pain here is broader and more diffuse and at a deeper level.

I do believe that there is an additional migration to the same purpose of attempting to "set up a new shop".

In a similar vein, the results are favorable @ this point. The power setting was on high.

3. The third target was the right thyroid; again this is understood to be completely experimental and any results will need to monitored over time when US imaging resources are available.

What is noteworthy is that on high power there was an immediate gagging / coughing reaction that took place w/ very light pressure of the probe upon the thyroid region.

A deep-side interaction of the US energy with the thyroid is definitely taking place.

I backed off immediately from the high power setting.

A similar but reduced reaction took place with the medium power setting. I also backed off immediately here.

A low power use for ~15 min produced no observable effect or sensation. This will continue to be monitored.

4 The final application was to the right shoulder on high power. There has been remarkable improvement in the shoulder w/ the use of US over the last couple of months.

The first trial w/ the particular US device produced an identical very sharp pain (identical to that now experienced on the left hand knuckle) w/ the use of high power and was also not tolerable. A definite pain center was located there, and but also appeared to exist at the lower level. Low & medium power was undoubtedly used.

The second use of US (likely a couple of weeks later) w/ a high power setting produced only a mild level of pain and US use on the right shoulder was then highly tolerable.

Since that time, condition have only improved with the mobility and strength that has now returned to the right arm and shoulder. Residual mobility issues do exist w/ the

right shoulder, as it appears did sustain some significant injury over my lifetime, ie,

1. a 12-13 ft fall approx 10 years ago.
2. Excessive hard ax use for many years in the surveying profession.

There seems to be little doubt that US is having a significant beneficial impact * by reducing the pain level and "residency" of the microbial networks (presumed to exist) w/in the body.

Now let's return to Tube 4-1 (Dec 24 2018)
(We must very much anticipate combinations / mixtures)
Absorbance Peaks.

s 3346 - 3256 Polymeric alcohol
recall that amides require carbonyl (not present) [thioamide excluded]

Hydrocarbons in general
m 2932 Closest to $>CH_2$, methylene

m ~~2980~~ 2890 - Closest to methyl

s 1014 Organics not a great match
Closest match is phosphate PO_4^{3-}

m 1342 NO_3^- is ~ 1350.
~1340 is alkane (Parker) - assign this

s 633 ~650 C-Br, strong, bromo derivatives
570-705 C-S thiol, sulfide, weak absorption

w 1655 1658-1648 terminal olefin $RR'C=CH_2$ C=C
med absorption 1662-1652 cis. olefin $RHC=CHR'$
Weak absorption, does not satisfy carbonyl (1600-1820)

m 774 Not clear
770-730 C-H unsubstituted phenyl
840-750 C-O epoxide

It is difficult to assign any new functional groups here.

The most interesting groups I see here is the olefin presence, and the presence of C=C.
I would, however, anticipate alkene absorption in our case (i.e., > 3000 cm^{-1}) and we do not have this (3040–3010 cm^{-1}). Parker explicitly references the olefin in this range.

Looking very closely, we do indeed have a slight disturbance (absorption) exactly in this range.

This does, therefore, isolate the case for the olefin. There appear to be no strong competitors w/ the absorbance @ ~1655. We also notice that our original absorptions here is weak.

The RCH=CHR' structure is therefore fairly strongly indicated here.

What are these types of compounds?

* Olefins are polypropylene, a synthetic fiber

Next we look @ Tube 4-6

Absorbance peaks @:

1610-1540 (vs) $-COO^-$ Carboxylate

s 1545 RCO_2^- or the inorganic side (Parker)
Secondary Amides (1510-1550)
Secondary Amide (1515-1570) No other real competitor

s 1419 RCO_2^-, HBO_3 Parker Inorganic
1400-1460 $-COO^-$ Carboxylate
1440-1350 S=O RO_2SO_2 sulfonic ester

m 1102 SO_4^{2-}, $P_2O_7^{4-}$ Inorganic, Parker
~1120 Thioamide $-NH-(C=S)-$

m 662 ~690 C-H $RHC=CHR'$

Notice we may show the presence of an amide but once again no Carbonyl group.
Be on the lookout for the inorganic amide once again.

Notice that we do have absorbance @ ~3500 ATR weakens the portion of the spectrum.

~3520 is a primary amide N-H (strong)
~3500 is a primary amine N-H (medium)

I must say, we once again have a reasonable case for the postulation of a

Thioamide. This is a very troublesome prospect.

Another observation on potential therapies:

It appears to me that the methyl salicylate balm has also been very effective. I no longer notice or sense a localized congestion/cough from the upper right chest/shoulder region. There appeared to be a definite improvement here as it was combined w/ ultrasound treatment. My assessment is that this location has chronically hosted a network & biofilm center which is where any cough seems to originate & reside.

Summary: Both ultrasound and methylsalicylate
* appear to be effective, noninvasive therapies against CDB network formation w/in the body, at the very least potentially so.

Mar
Dec 12 2019

I have finished Tube 4-6. Now to Tube 1-2 (Dec24)

One spectrum is relatively simplified with absorbance @

S 3211 (notice ~~bbbbb~~ sharp intense peak)
Amine Salt is (3145-3355) (Solid) 3300 is
Primary amide is ~3175 polymeric OH

S 1644 Carbonyl looks strong this time
Notice Para amides are the carbonyl candidate (i.e. ≤ 1690)

S 1058 Inorganic HSO_4^- Parker
1058-1053 C=S $(RS)_2C=S$ trithiocarbonate
1030-1090 P-O-C Phosphoric ester

S ~600-
705-570 Thiol, sulfide
This is weak however, does not fit.

The remains a question.

We have a strong case for assignment of an amide here. This time we have the carbonyl group without question so the means that we do now have the protein,

We also have a case for the presence of sulfur here.

Therefore we make the case here for a sulfur based protein.

Furthermore, we have a lot of historical evidence for the existence of an iron-sulfur protein (ferredoxin).

Thus is the most probable assignment here, that of an iron-sulfur protein (ferredoxin).

Let's look @ the conditions of the separation.

This looks to be a case of using ionic salts for the separation eluate. $MgSO_4$ was involved here and notice a blue-grey color was formed in the tubes. Note also the difficulty in removal. This color may correlate w/ the protein (first separation) separation of many years ago.

—

Now we go on to 1-5, 1-6 where acetic acid was introduced as the eluate.

We can see that both Tubes are the same compound. I will average these two spectra.

Our absorbance peaks are at:

S 3312, 3212 NH bonds seem to be dominant here.

Notice no carbonyl (1600-1820)

S 1556 Inorganic (Parkin) RCO_2^-
~1570-1515 Secondary amide (solid)

S 1419 Inorganic RCO_2^-
C-N 1418-1400 primary amide
~1410 aliphatic amine

S 1089 ~1120 Thioamide

S~616 705-570 C-S
Thiol, sulfide

All signs are once again of the thioamide.
This is the third repetition of this compound assessment & assignment.

* Our note for Dec 26 2018 must now be considered very carefully.

April 12 2019 Percha Dam State Park, NM

Additional Health Comments:

There is little doubt that the "network" (ie, biofilm, filament, quorum sensing network) can migrate through the body.

All appearances are also that it favors joints and/or weak or vulnerable points in the body, such as locations of previous injury.

Ultrasound and methyl salicylate balm both most definitely seem to provide benefits in pain reduction and pain elimination.

I have ~~great~~ made great progress in the chronic pain location that has resided in the right shoulder - upper arm during the past several years, along with the chronic ~~bo~~ lung congestion - cough over this same period. I attribute the pain and cough reduction / elimination specifically to the use of therapeutic style ultrasound (1 MHz signal) and the methyl salicylate (wintergreen) - cocunut ~~bo~~ oil balm that is also under repeated use over the last couple of months. The methyl salicylate balm ~~is~~ approximately 10% by concentration. Both ultrasound and methyl salicylate appear to somewhat equally beneficial, but ultrasound may have an upper hand the deeper in the body the pain center resides.

It seems fairly certain that the nerves are being affected (negatively and painfully), especially in combination with the joint issues.

Here is an important observation:

As progress was made in the reduction of nerve/joint pain in the right shoulder,

MOTION, ie, migration of the pain center to new locations WAS APPARENT.

Specifically, new pain centers appeared rather quickly. One center developed in the left hand, first finger knuckle and a second strong center appeared in the right knee. The knuckle appeared over a period of ~ 2 weeks in a gradual fashion until it actually began to restrict mobility. After it became known that the pain type (ie, nerve and joint) was of the same type that has been ~~present~~ ~~cho~~ chronically present in the right shoulder, ultrasound and methyl salicylate balm were immediately deployed.

Benefits were immediately noticeable within 24-48 hrs with consequent pain reduction.

As progress ensued w/ the knuckle activation a rapid shift / development of strong pain in the right knee developed. The development was sudden, within ~ 24 hrs and immediately hindered my ability to walk.

On the surface, it appears as though an immediate flare of arthritis has taken hold however, the suddenness made no real sense.

Both knees were affected to some degree, but the right knee pain was certainly the stronger of the two. The pain type was again identical, nerve & joint pain combined.

Once again, ultrasound & methyl salicylate balm have been extensively employed. The more serious nature of this movement modality has escalated my attention to the matter. Treatment has continued over the last month.

Once again, tremendous progress appears to have been made with the use of ultrasound & methyl salicylate balm. I continue to pay very close attention to the matter.

My assessment is that the network continues to seek a location to "set up shop" and establish residence in the body.

It appears as though ultrasound & MS balm are both presenting serious obstacles to that development, but that nevertheless a very very serious struggle and challenge continues to take place.

I do think that w/out ultrasound & the MS balm that has been developed/discovered that I would be at a serious disadvantage.

It is my hope, of course, that I can retain the upper hand in this "battle" but of course there is no guarantee of this.

I would say that the network is quite persistent, tenacious, and relentless. The mobility and migration of the network in the body is a primary & paramount attribute of its ability to adapt & survive.

None of the cores or any surprise hand upon what is known about the ability of the particular microbe to survive under hostile & adverse conditions.

the best that I am able to do @ the point in to chronicle my trials, my successes, and my failures.

It is most dependably, very serious business at hand and the time is without doubt, one of survival.

It is known that the microorganism is destined to survive and adapt @ the point; it is questionable to attempt to claim such optimism for the human species, at least w/in one lifetime.

I do feel fortunate, however, that it has maintained my state thus well, thus far, and the long. Many, many other have not fared so well, and it is for the that my work exists. I can only hope that this work will be made available to those in need that may benefit from it.

—

Next, we need to start talking about the thyroid to greater extent.

There is little doubt that I (and I think most others) have a problem here.

The simplest and most obvious indicator here is that of

Body Temperature.

The 98.6 "norm" has now been lost decades ago. I will contend that the vast majority of the human population on the earth show low chronic hypothyroidism, and subsequently a lower than "normal" body temperature.

Thus, I will also contend, is due largely & specifically from the health effects by the "Close Domain Bacteria" (CDB).

We are living a duality here; the norm of 98.6°F Continues to be stated, but it is seldom to be found in practice now.

The "norm" will require an adjustment and gradual phase-in, of this there can be little doubt.

"Welcome to the New Norm".

The phase in, in both literature and conversation, is required to be both subtle and imperceptible, such that it does not draw too much attention.

I, like most others (as I contend) indeed have the problem of low body temperature. I have worked to alleviate it in previous years (iodine therapy) but it seems to have reemerged more strongly over the last couple of years or so.

It appears that a drop of ~1°F in temperature can be sustained if relatively minor but, even though it is MOST CERTAINLY less than ideal: ideal.

Recall that " every biochemical reaction take place at a specific temperature and pH".

There is most certainly a detrimental effect from any departure from the norm, but we are only a matter of degrees.

When you get to 2-3°F below normal, you have a problem.

I have found myself in that range more than I would like to admit in recent weeks, once I have started testing

for the activation again.

The testing was prompted by symptoms of:

1. Continued weight gain, even under conditions of decreased food consumption.

2. Constipation, or decreased bowel movements.

3. Poor digestion in general, with excessive enzyme and alka-seltzer use.

4. Joint pain

5. Sustained, or chronic fatigue. Especially alerting to the condition of not feeling rested even after sleep.

6. The napping periods slowly continue to increase in duration. At one time, several years past, 20 min naps were usually quite sufficient for reinvigoration; Now 1 1/2 - 2 hr naps are more common. I do, however, also usually run a double shift!

1. ~ 8AM - 4 to 6 pm. awake

2. 1½ - ¾ hr nap sleep

3. 8PM - 2AM (or 3AM), quite commonly. awake

4. Up @ ~ 8AM to 8:15 AM

Total sleep is therefore ~ 7 - 8 hrs total per day (6½ - 7) so nothing is actually unusual there. But I usually do require a split shift, as mentioned.

The symptoms listed are classic hypothyroidism. My temps were frequently down in the upper 95's range. That is far too low.

I have since started w/ iodine therapy again. It appears that benefits may also flee very readily w/ the change, and much more rapidly than I had surmised. I am much more frequently seeing temps in the mid 97's now.

I am more commonly ranging from 96.5 to 97.5 already, even under a shot treatment period. I have also noticed substantial improvement in the symptoms noted.

The iodine strategy is very much actively in review @ the time, and all signs are favorable @ this point.

Recall also that I am now known to have a 1" adenoma in my thyroid.

We're different, however, in that I am having to aggressively and actively employ numerous somewhat unusual strategies to maintain reasonable health.

I suspect that I would be @ a major disadvantage w/out the ability to apply these strategies, and I now consider them all to be paramount for review in association the CDB complex.

The list now include:

1. The many supplements, which have been written about in numerous research papers. It is difficult to keep up fully w/ that regimen and extensive list.

2. Ultrasound therapy - fairly common & regular use Bakey plate in the nerve - joint pain centers

3. Methyl Salicylate balm use - also quite regularly, and to the heavy, as observed.

4. More recently, the re-employment of Iodine therapy, in an effort to counteract Compensate for a potential increase in hypothyroidism.

May 11 2019 – Monticello UT

Back @ the lab for approximately 1½ months.

Continuing w/ the health observations:

There are four locations of persistent incident pain. It now seems to be established that joint-neural locations are primary targets for inflammation & pain.

The four areas are:

1. The right shoulder – upper arm
2. The knuckle on the left ~~right~~ hand index finger
3. The mid back on the left side & lower left shoulder (very difficult to access)
4. The knee, with the emphasis upon the right knee.

There has been movement between these four centers over the last several months, with the latter two increasing in severity and persistence, and the former two weakening.

The primary treatments are now ultrasound & methyl salicylate liquid (~15%) (i.e. wintergreen oil). These combined appear to be a very effective counter strategy to network neural development & inflammation, but patience and persistence in application are both required.

The mid back, lower shoulder is an interesting point of vulnerability, especially in light of the defensive movement of the network upon the body. This is an especially difficult region to access and thus an extension tool has been devised for application of the balm.

Iodine treatment and temperature monitoring are also important strategies in place. Approx 5 drop bottle in water for internal use & external application of 10% Betadine on the right throat-neck region. There seems to be little doubt that the iodine use have produced immediate benefits in lowering the body temperature ~ 1 to 1.5°F. Temperatures were in the 95.5-96.5 region - this is when serious chronic problems began to develop (eg fatigue, constipation, joint pain, weight gain, etc).

After application of & use of iodine on a daily basis, the temperatures are now more likely to be 96.5-97.5°F.

The degree use is extremely important with respect to thyroid & body function and regulation.

The chronic ear infection (this had become quite troublesome) has been largely relieved or eliminated since the use of iodine bugan. There is little doubt that the mild increase in body temperature is @ the heart of this relief & improvement.

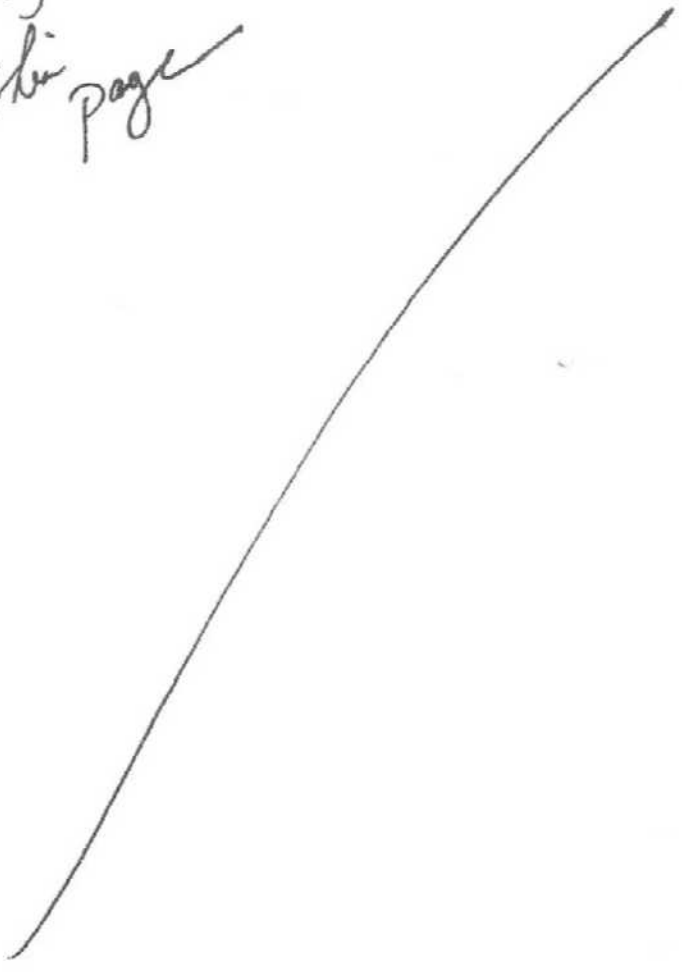
The ultrasound can still only be used at the medium setting in the right knee. The situation in the knee(s) has improved dramatically since employing the ultrasound - methyl salicylate strategy, however, this is a deep seated assault w/in the body & additional patience & persistence are expected for some time yet. The situation, in general, appears to continue to improve whenever & wherever those methods are applied.

In addition, supplements involving Vit D (8000 units daily), collagen, Vit C & B complex have also been included w/in the ~~tra~~ treatment methods. These also appear to be of assist.

I intend to use ultrasound imaging (separate device) in the near future to see if I am able to determine if any change has occurred in the thyroid cyst that is known to exist from the ultrasound examination done professionally during this last winter during AZ travels.

May 14 2019

Disregard this page



May 17 2019

I have been working on and developing methods in column chromatography.

I am working w/ a colored dye (RH) for development reasons. Testing methods is much easier w/ color as a reference.

I have seen that acids & bases seem to be as effective, or often even more effective as the eluent in the column. This is the method of ion exchange chromatography.

Methods involving polarity sometimes work, and other times have no effect. The combination of both methods is often quite productive.

The problem is that acids & bases appear to be highly destructive to the alumina substrate. Many column constructions have been sacrificed during the various trials underway.

I am, as a result, experimenting w/ a silica based column, i.e. white fine sand as the stationary phase. It may be quite constructive & not subject to damage from the acid-base situation

@ this point, this idea seems to be working superbly and the first separation has taken place (dark blue) with water as the eluent.

The pace of the drip is now also quite steady and strong. We now shift to alcohol (isopropyl) again to introduce a higher polarity.

Isopropyl alcohol is bringing out the red layer almost immediately. This is also excellent. Very fast & uniform descent of this layer.

The red layer has separated w/in 5 min. Absolutely superb.

What appeared to be reddish in the column is a strong purple as the eluate.

We now have a remaining shade of purple in the column to be removed with KOH. Ethanol did not remove that tint, neither did acetone (acetone is damaging to probes) & therefore increased polarity shows no benefit.

It is here that we move to the acid base, ion-exchange approach.

Also the increased air pressure pump is also working superbly.

The different colors are most likely due to different transition metals. You would likely need to attempt electrochemistry along w/ an educated guess based upon the color present

1. rich blue
2. rich purple.
3. milky gray purple

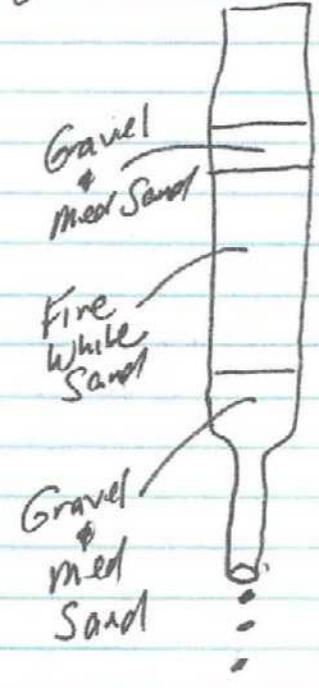
Now w/ KOH we once again have an immediate separation / descent of the next layer
We are getting some precipitate, hopefully it will not damage the column

There remains an additional layer after NaOH.
Light blue w/ hints of red within.

Ethanol had no affect.
Try vinegar next

Vinegar – no change.
Some activity w/ ammonia.

Very successful separation is taking place w/ ammonia.



May 10 2019

At this point, the sand (SiO_2?) column has worked superbly and it has the major advantage of not being torn apart w/ the use of acids & bases, which then allows us to use ionic exchange methods in addition to polarity methods.

It seems difficult to want to return to the aluminum column w/ all the problems that appeared.

Now let's reconstruct the smaller diameter column w/ the fine sand & gravel/sand endpoints, as it should have less resistance: Approx 4" column w/ 1" gravel/sand endpoint caps appear to be a suitable dimension.

The large column, approx 1" diameter, also did work extremely well. It will generally be slower, however, than the 1/2" column.

I also see now that I do have a very large bag of silica gel available, also undoubtedly in planning for such work.

Six inch ½" diam Column developed w/ sand:

I am getting a better separation here. I am retrieving the leading green layer prior to the red blue layer that follows it. The amount of sample retrieved is normal, however.

Water is the eluent.

Not the red blue pigment has come through well the work is looking very good.

May 19 2019

Water as an eluent has completed its cycle. Three separations took place w/ the RIT Navy Blue dye

1. Chemical blue (small sample)
2. Red blue (moderate sample)
3. Light blue (large sample)

Isopropyl alcohol now in use (necessary polarity level (Acetone never recommended however as it is destructive to probe & plastics). A red layer is forming and very gradually descending the column.

I have a nice even, but small red layer gradually descending the column w/ the use of isopropyl alcohol.

Calcite, fine sand make excellent column material. Capped on top & bottom w/ gravel & medium sand.

Perfect separation of a red layer has taken place.

The milky purple layer has been extracted w/ the use of KOH.

Notice that the use of ethanol was skipped (increased polarity). This is debatable.

Notice also that ammonia was used prior to KOH. This is also debatable.

The KOH does not seem to have damaged the column, but it is possible that it did react with it in some fashion to form the milky purple eluate.

Ammonia is now being used. No immediate effect is apparent. We have purple & blue that remain throughout the column length.

The column is in an extremely slow state w/ no apparent color separation taking place with ammonia, but the column is not plugged.

I am surmising that the KOH reacted w/ the gravel & sand in some fashion to create a soluble carbonate form. In the case of alumina, it seems as though it destroys the column; in the case of sand there is no apparent damage to the column.

Five distinct layers from the particular dye lot now already been separated: blue-green, red blue, light blue, rose red, & milky purple.

May 20 2019

I am now actually picking up some separation w/ the use of ammonia. There has been a delay of ~ 12 hrs for the effect to take place.

The column was essentially stopped (plugged) for ~ 12 hrs and now it has opened up again in a fairly slow mode.

There are questions whether the sequence of solvents used make a difference in the elution; I am fairly sure that it does.

I think that it may have been counterproductive to bypass the use of ethanol and ammonia before proceeding directly to KOH.

I am fairly sure there should have been used to make the progression of extraction more gradual. Strong acids & bases should be used as last resorts in the chain of polarity & ionic exchange series!

I am also fairly sure that I should also use weak acid & base before proceeding to stronger ones.

Stereochemistry

Polarity	Ionic	
Water	Ammonia	
Is. Prop	Vinegar	
Ethanol	Weak Base	KOH
Acetone is bad news for proteins	Weak Acid	HCl
MEK, etc	Stronger base	KOH
	Stronger acid	HCl

I have now added vinegar acetic acid
It appears that we have some activity developing. A purple layer appears to be separating from the overall blue color spanning the column. Now the question is will the layer separate and descend? The flow has changed to smaller and slightly more frequent drops, so this is favorable. Acetic acid is also causing some bubble formation @ the entry point - we see that in general acids & bases are harder on the column substrate and will hold up better, I believe.

An interesting information source has become available & known:

Basic Liquid Chromatography; Nina Hadden, 1971

from my library. On Page 6-11 there is a table of specific frequencies for different Chromophores (essentially functional groups) in the UV range.

I have never seen such a chart that is as helpful with UV interpretation & spectra as this.

Most of the entries are below 220nm so this reduces its utility, however it can still have some value.

I have scanned Lysol's iodine as a first test of this chart. The UV spectrum is quite active, with peaks @

351nm
288nm
233nm

In Hadden's book we have Iodide given @ 260nm. So I can't say we have a match there.

But it will be interesting to keep an more on future UV analysis.

Next, the use of vinegar in the column has dramatically increased the flow rate.

It seems to have removed a residual blue layer but no real upper separation of 2 distinct layer. What it has done is leave a uniform purple color that spans the entire column.

We see a similar purple from a previous run of the column. The question now is what solvent is going to extract that color?

There is a fair amount of foaming that is occurring at the tail of the column. The flow rate is relatively rapid now.

Ok, just as I was suspecting.

The order of the solvents has a MAJOR impact upon the elution.

This is a significant discovery.

Not only
1. Polarity
2. Ionic Exchange

but the order of the solvents used is critical to the result

The were discovered by the following means.

1. The earlier use of ammonia did not produce any dramatic singular result, however, we were able to extract one layer w/ the process.

2. Vinegar was tried and produced a marginal layer but it did dramatically increase the flow rate on the column.

3. Ethanol was used and no effect whatsoever was observed other than maintaining a fairly rapid flow rate.

4. Then, ammonia was put on again, AFTER the Vinegar and Ethanol had been used w/ marginal results.

VOILA! Immediate and dramatic results.

The time the ammonia is producing a clearly descending unspoiled layer, as well as leaving the column perfectly clean behind it.

This is the perfect solution to ending the Column run.

The flow rate has now once again decreased dramatically mid-stream in the layer elution. I anticipate that it will still decrease in time.

This shows us that the ORDER of the eluents is as important as their polarity or ionic state.

Three major variables affecting the success of any run have emerged with respect to the use of Column Chromatography

1. Solvent polarity !
2. Solvent Ionic State !
3. The order, or Sequence of solvents used !

This is the information that I was after.

A red dye can be used as an indicator front. I might try this @ a later time.

Since the column has mildly stalled out @ the bottom I have added a little vengeance to see if I can increase the flow rate.

Strong KOH has cleared the column fairly well all purple has been removed. There is a tinge of red that remains in the column. Very weak but detectable.

May 21 2019

Setting up another toxicology study today.
I will be using brine shrimp & the soluble protein.

Since brine shrimp require salt water, we will incorporate the same control into the protein solution, ie 25 ppt brine solution.

Lets prepare a control brine solution,
Pink salt used as in the brine hatchery solution

0.5% Protein in 50 ml H_2O is 0.25 gms.

Beaker mass: 35.61 gms
" " w/ spatula: 47.41 gms
Spatula mass = 11.80 gms
I have 0.22 gms protein evaporated
25 ppt = 1.25 gms salt in 50 ml H_2O

We have made therefore an approximate 0.5% protein-brine solution. Actual: $\frac{.22 \text{ gms}}{50 \text{ ml}} = 0.44\%$
The will be diluted even further as it will be added to brine shrimp that are already in pure brine solution.

First ~~toxicology~~ toxicology study is complete.
Significant neural & motor disruption in 10 shrimp w/in 1 hr.

Estimated concentration of solution 0.1%.

Now we increase concentration of protein solution to 1.00 gms in 50 ml brine solution

= 2%,

This in turn will be diluted in the microscope sample to ~0.5%, or similar to that used for plant & protozoa studies.

This study was completed w/ total success and it has been presented within the research paper entitled:

"Margellons Toxicity - A Continued Report"
May 2019

May 22 2019

The column trial w/ the clue has gone extremely well. I have approximately six separations that have been made and the column is almost completely clear.

There remains, however, the slightest tinge of a reddish color in the lower half of the column.

Now, how to extract this? I have used
1. polarity
2. ion exchange
3. sequence of solvents

] to effect separations

How can I now remove this residual?
~~The~~ One remaining solvent is that of a strong acid. With alumina, it destroys the column. Well, how about our sand column? Let's have an adventure.

OK, the HCl appears to be destroying the gravel, but not the sand. There is a very good chance that this is enough to undermine the column w/ damage or destruction to both gravel/sand end caps to the column, top & bottom.

Lots of bubbling, I assume the gravel is limestone now. If I am very lucky the sand will maintain its integrity.

I love to wonder if my glass beads I have will be superior. Go end cap me along w/ sand or SiO_2 when gel as the column substrate.

The HCl is being pretty hard on the top endcap. It is slow also affecting the bottom gravel / sand endcap but @ the point to a lesser degree

I think that it would difficult to destroy the endcaps because of the medium sand that has been placed there. Damage looks like it is confined to the gravel and the last suggestion is to replace that w/ glass beads on the next packing.

With alumina acid reacted with both the gravel and the alumina and then repeatedly destroyed the column even when using weak or organic acids such as vinegar.

The partial reaction appears to be causing no real harm to the column.

OK, it did break down my column. Not as bad as before but it does need to be rebuilt.

May 23 2019

Since the column was damaged, a test has been run with (to me) surprising results received.

The test is that of strong acid (HCl) directly upon sand in water in a test tube.

There is, in fact, a vigorous reaction between sand & HCl; this is unexpected on my account.

This explains why the column is being damaged w/ strong acids. Strong bases seemed to cause no such damage.

Now let's see if ~~sand~~ HCl affects glass beads.

Ok, it does not — GOOD.

I am also running a test w/ Silica Gel and HCl.

It also does not react. — GOOD!

This means we want some very fine glass beads to help form the sealing caps on the ends of the column.

Actually there is a minor reaction w/ silica gel but it is nothing like that of sand.

I have, therefore learned some things through trial and error on Chromatography Column Construction:

1. Silica gel appears to be the most impervious material thus far. And appears to be preferable for its porosity, but strong acids are going to damage the column. Silica gel, unless we can acquire/obtain a more coarse grade, will be a slower operation but presumably more stable.

2. Glass beads are impervious to the HCl and are therefore required for the end caps. A combination of coarse beads and fine beads is required to make a proper seal.

3. A smaller column (~200 mm - column only, not reservoir) w/ diameter ~ 13 mm seems to be the ideal size for the lab. A 6" silica gel column appears to be more than adequate.

4. Avoid strong acids if at all possible. Silica gel & glass beads seem like it will hold up quite well, but try to keep the acids/ as weak as can be effective.

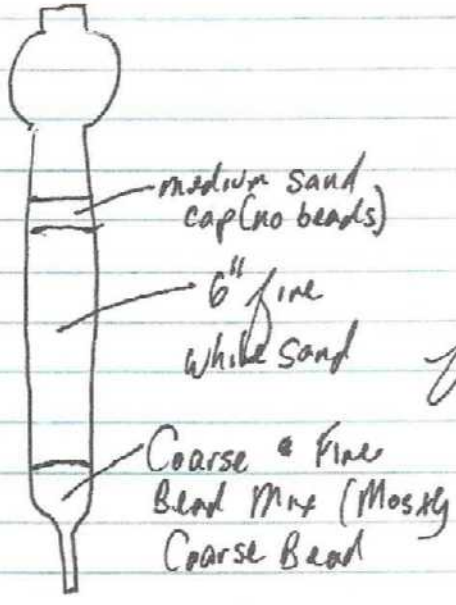
5. Only moderate suction & flow heads are required for the top protective cap.

One problem that I am seeing, however, is that the silica gel appears to be very slow. This could be a limiting issue.

If you could

1. Stay away from very strong acids
2. Construct the bottom cap of mixed coarse & fine beads
3. Construct the top cap of medium sand alone.

The arrangement could end up wordy quite well and would still allow for more fine sand to be added w/ a new coarse sand cap should it become partially damaged.



The arrangement offers the best compromise. It has a reasonable flow rate w/ moderate suction. Minimize the use of strong acids. Damaged fine sand can be easily added to and new medium sand cap added.

Watch for any coarse beads falling into the vapor flue - you do not want this

OK, two columns have been prep and in the fashion, they look to be preparing well.

Solvent Candidates:

Polarity	Acid	Ionic Enhancer Base
Water	Acetic acid	Baking Soda
Isopropanol Ethanol	Citric acid	Ammonia
No Acetone	Dilute HCl	KOH - NaOH
MEK	Dilute H_2SO_4	
Octane - Xylene	[Salts?]	

And the ORDER with all of these concentrations can make a difference.

May 24 2019

A couple of questions have arisen about understanding the presentation of data on the Morgellons Related Project (MRP) study.

The need @ this point is to simply be able to retrace the steps of the data.

First off, analysis notes seem to be in two main sections of the original laboratory notes:

1. Volume 10 Circa Aug 23 2015
2. Volume 15 Circa July 24 2016

Some things that I see:

1. Total no. of people that participated in the short serial questionnaire appear to be 971.

2. The number of people that answered any particular question within a particular body system set varies from this.

3. For instance, on the Skin and Nails section, 354 people in total answered one or more questions (but not necessarily all questions) within that particular body system set of questions.

Of those 357 people, 60 people

~~Responded TO THE QUESTION (we~~

~~do not yet know how they responded, we~~
~~yes or no)~~

RANKED the issue (symptom) of materials

or substances emerging from the skin.
It was not a yes no response, it was
a ranking.

therefore of the 357 people ~~than answered the~~
~~skin~~ that participated (we dont know
how many questions they answered)
60 of them ranked materials emerging
from the skin as the most common.

This was an analysis of variance problem
that was set up for the MRP results.
All is fine here.

May 26, 2019

— Now, on the Chromatography column. There is another good demonstration of the importance of the ORDER of use of solvents that is taking place.

We are showing repeatability but it requires keeping track of the order of solvents used, in addition to the polarity and ionic exchange state.

Early in the column run, ethanol was used immediately after water was used as the initial solvent. Both water and ethanol had no effect. Strong KOH was used to very slowly (2 days) bring out the first deep blue separation layers)

After two layers were produced, rich blue and light blue, with the use of KOH, ~~ethanol~~ isopropanol was then used (~~attempted~~) again.

Under these conditions, ie post KOH, a vivid red layer separation is produced, even though (ie, alcohol) ethanol produced no such result on the early portion of the run.

Our sequence thus far is now

1. Water (no result)
2. Ethanol (no result)
3. KOH (dark blue layer)] two day
4. KOH (light blue layer)] separ
5. ~~Ethanol~~ – vivid red layer (actually red-purple)
   Isopropanol

6 Ammonia
   From a uniform purple layer throughout the column, the addition of ammonia AT THIS STAGE creates, once again,

a dark blue layer which once again descending steadily through the column.

Clearly the order of the solvents would be a critical factor in the separation process in addition to polarity, acids, bases and salts.

We have not even investigated the use of salts yet but we do know that precipitation of salts is a serious issue that can destroy the columns. Caution is advised here.

I would like to have a refractometry as a detector and we also know that UV detectors, especially a scan UV, are a marvelous detector across the board.

Refractometry:	Solvent	Brix		
1st Layer - Dark blue	KOH	0.7		
2nd Layer - Light blue	KOH	0.1		
3rd Layer - Red - Rose	Isoprop	21.1	~~Ethanol~~ Isopropanol	23.7
4th Layer - Dark Blue	Ammonia	12.1	Ammonia	6.0

Paco spectrometer measures from 380-950 nm. Not bad as we also pick up some NIR. Also it detects fluorescence @ two wavelengths.

The says that we have some excellent portable detectors available to us now as well as a fantastic separation method. CONTINUED → VOL 25