CARNICOM INSTITUTE LEGACY PROJECT

A Release of Internal Original Research Documents

Authored
by
Clifford E Carnicom
President, Carnicom Institute

Laboratory Notes Series: Volume 25
May 2019 – Sep 2019

www.carnicominstitute.org

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

Volume 25

May 2019 — SEPTEMBER 2019

May 26, 2019 Monticello UT

I continue here to develop the methods of liquid Chromatography (LC) for the separation of compounds and also detection methods.

Portability is important here also for the significant time that I am now away from the primary lab.

I am attempting to develop portable methods of

1. Compound separation (LC)
2. Detection of colorless organic compounds
    1. (UV) – VIS (NIR) spectrometry
    2. Fluorescence
    3. Refractometry
    4. pH
    5. Conductivity
    6. ORP

    [all portable now]
3. Compound identification methods
    1. NIR
    2. Electrochemistry
4. Titration methods

1. The lab you need to acquire:
    1. Mid IR
    2. Full UV & NIR in solution to 1100 nm
    3. Gas Chromatography
    4. Osmometry

I can already see that refractometry will be a simple useful detection method. Controls on solvents will also be easy to acquire.

My next topic of interest will be to look @ the extinction separation in the range of 380 - 950 nm (the range of the portable Paico spectrometer) that is far away from 400 - 700 nm, assuming color-less separation only.

From previous note of May 26 2019:

	Solvent	Brix
1. 1st layer - Dark blue	KOH	9.7

We do have some absorbance here < 400 nm and > 700 nm. That is good.

Result: There are some differences in the scans in the limited range of 380 - 400 nm & 700 - 950 nm but they are not dramatic. The red layer does show up as different, but this could also be due to the alcohol content. More work here would be required. A combination of methods

pH, Conductivity, ORP, refractometry - UV - [VIS] - NIR data would be used to effectively determine the level of difference between separations

I also want words of NIR can be used even through a cuvette, adopting a H_2O cuvette as the reference blank.

I basically have 3 blueish layers separated by these three different solvent ratios & a question is how different are they? We know that the red is different & logically all blue layers are different also.

But it would require further molecular analyses (electrochemistry, NIR) to determine that.

The entire segment over the last couple of weeks has been one of method development, especially for that of the mobile lab environment.

Ok, it is now time for the real McCay. The next stage of work represents an important & adventurous attempt to advance the research to another stage.

The objective is to now learn something about the nature and/or structure of the more recently developed SOLID PROTEIN form.

* This represents a very significant endeavor w/ undoubtedly numerous challenges ahead due to the protein being of soluble form.

* One of our first goals will be to see if we can develop some method of dissolving into solution.

Our first step is to rinse the protein to make sure the solution has nothing in but the soluble form.

I have rinsed the sample twice.

Realize that we are starting here from Ground Zero with no assumptions whatsoever as to the is a pure form of the advanced culture growth.

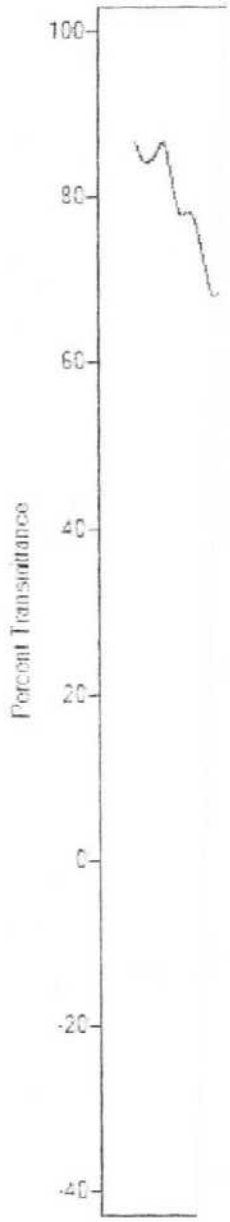
I have also taken a crude IR plot of the raw protein on ATR as only a starting point.

What do we see so far?

1. The protein is highly insoluble in concentrated KOH. It forms a dark brown precipitate.

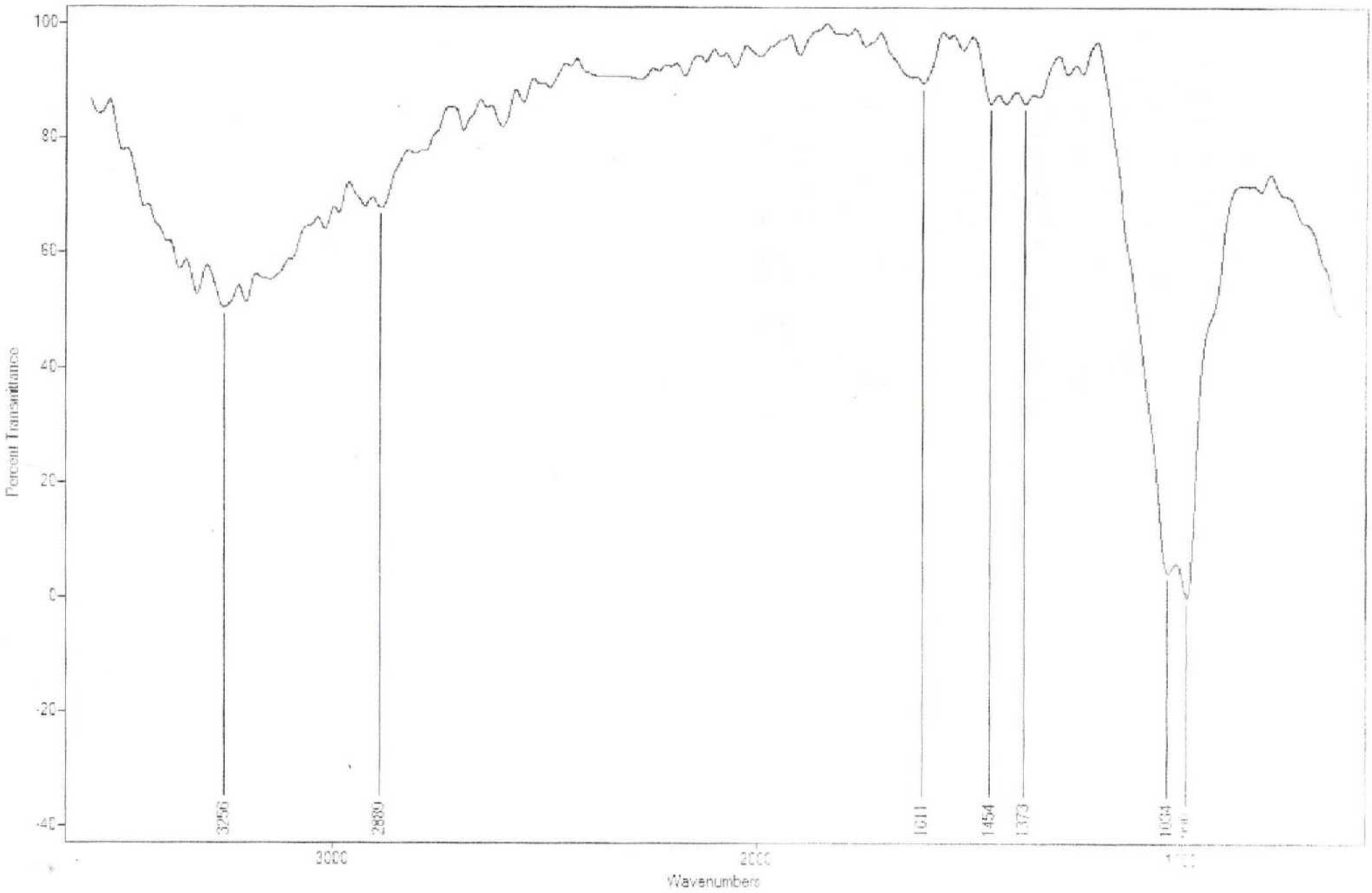
The spectrum is of limited significance - Preliminary Ob

CDB Solid Protein 2nd



printed on 05/25/20

CDB Solid Protein 2nd Gen Rinsed Only ATR May 25 2019 - 01.spc: 05/25/2019 16:29:57 title



FTIR

printed on 05/25/2019 16:47:44

In Concentrated HCl however, it appears partially soluble.

First lesson, be very cautious of introducing an alkaline solution into the LC Column with the protein. We must presume that it will precipitate and plug up the Columns.

I will analyze the limited information that is available from the IR & UV-NIR plots in the near future.

However, prior to this, I am making a dissolved/filtered sample of the protein after silylation but to Concentrated HCl.
It turns bright yellow in the process.
Iron Coordination Complex likely there.

Our filtered HCl protein extract look to be quite successful @ getting much of the protein into solution. It can also be shifted to an alkaline state w/out precipitation and only a mere Color Change to a light brown Color. It should be able to be neutralized for an additional IR ATR test.

Vol 17 Notes PP 284-286
Mar 22, 2017

Protein Colorimetric Test — Notes
Developed May 22, 2017

Vol 17 Notes PP 284-286
Mar 22, 2017 Page 298

Protein Reagent & Test Developed

Now
50 ml H_2O
200 ~~100~~ ml dilute dye
1 drop conc. NaOH - KOH (this turns it purple)
4 drops $CuSO_4$ - this turns it orange (clutter)
but with precipitate
add tartar to remove precipitate
(nice cherry red)

Now add protein - shifts to purple, very sensitive

A new protein reagent!!!

3 ml H_2O
20 ul dilute ½ & ½ red dye
6 drops 1M NaOH
1 drop 0.5M $CuSO_4$
trace visible tartar
Final Color is Orange, ~~not red~~ not purple.

200 ml H_2O
20 ~~+.5~~ ml ½ & ½
20 ml 1M = 2 ml 10M NaOH
3.5 ml $CuSO_4$ 1M
.75 gm Tartar

This works! Can detect down to 40 ul protein.
This is 1.38 gms/liter of protein.
Can appear to be a order of magnitude (~10) more sensitive than Biuret.
I suspect, eventually that I can detect to 1 gm/ml or ~ 1/1000.

This looks just right. You want the solution to be orange! If you add too much $CuSO_4$ it will color it. You have it very close soon

You might be able to dilute this by 50%.

BC300 Closest is 510 nm

You can dilute this reagent by 100%. and still get reliable results, sensitive to 20 ml = 0.75 gm/liter This is great.

Maximum Absorbance @ ~~580nm~~ 574nm 488nm.

Therefore the recipe is:

400 ml H_2O
2 ml 1/2 & 1/2 Rutz Cherry Red Dye
2 ml 10M NaOH
3.5 ml ~~Stock~~ 0.5M $CuSO_4$
0.75 gms Cream of Tartar

Easily detectable to 1 gm protein / liter

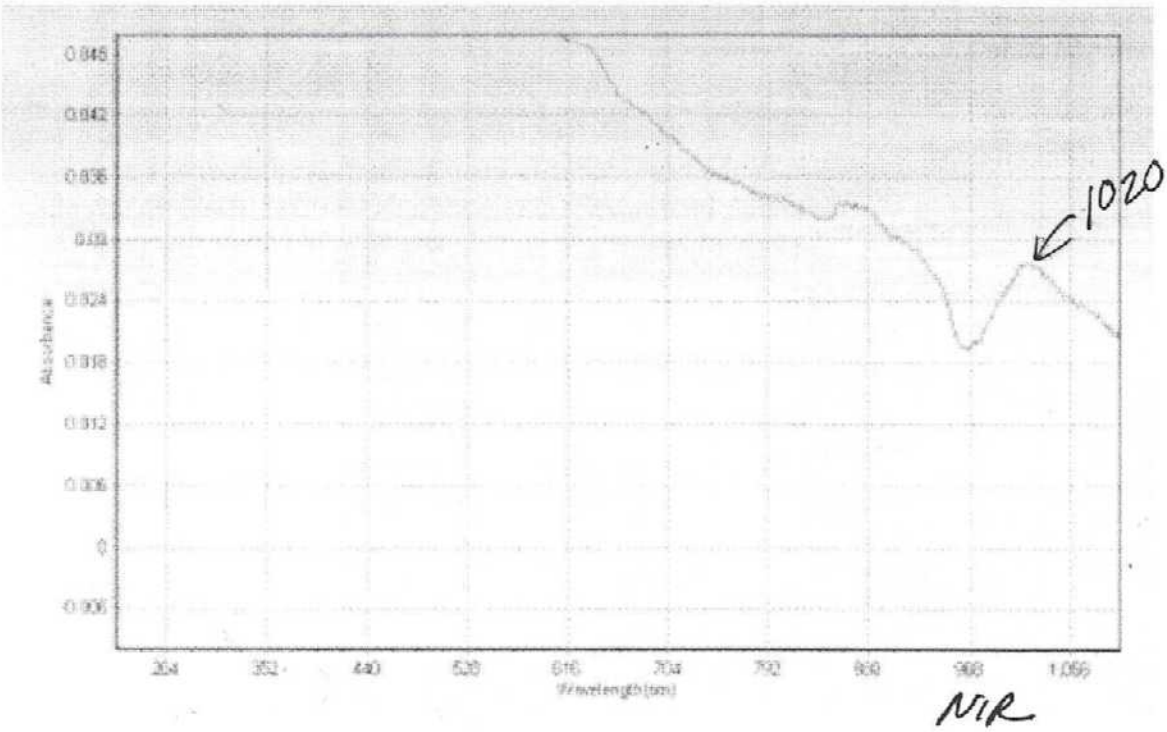
Wavelength of max absorption =

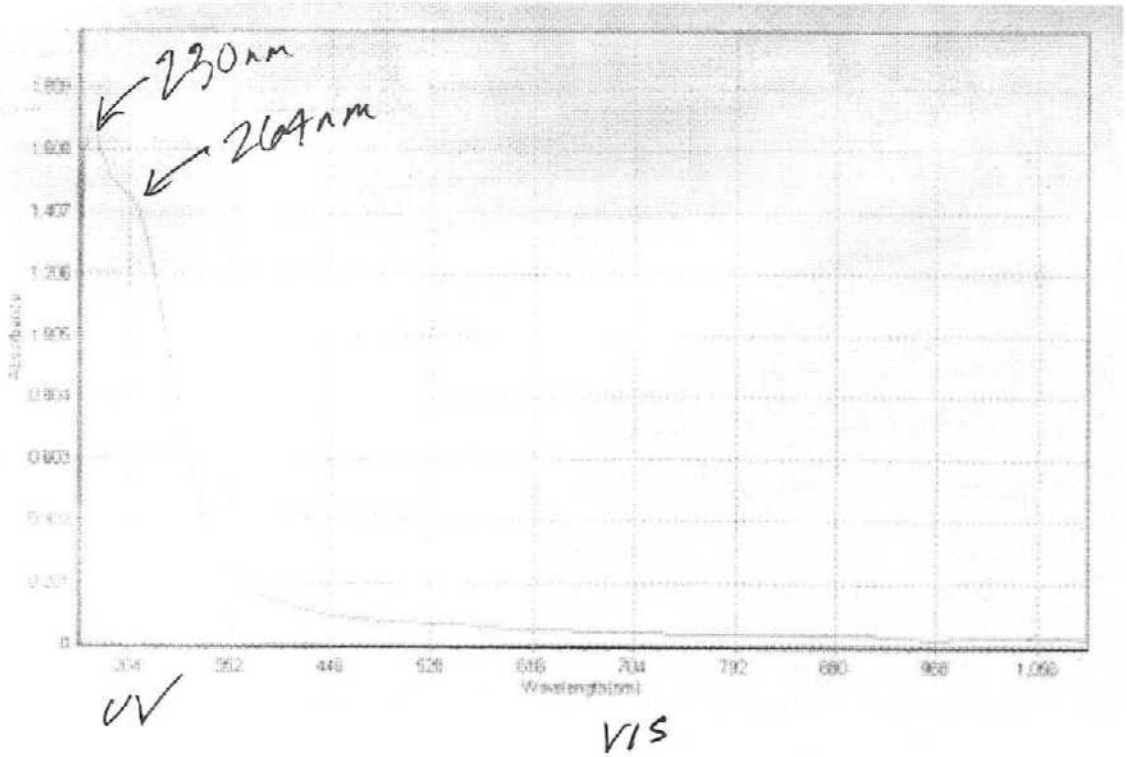
With Protein the reagent also has one peak @ 517 but it has another @ 534 nm.

The point of reactivity appear to be @ ~~534~~ 532 nm. This would be the preferred point of assay concentration. (On the BC300, 546 nm is the closest but Purdy can be used @ 546)

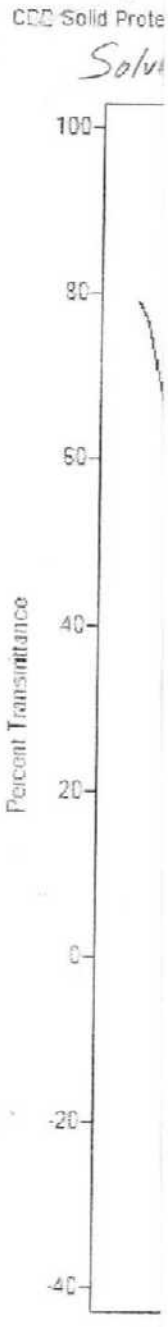
If ya look @ the spectra comparison the increase occurs definitely in the 530 region, which is in the middle of purple. In the reagent orange + purple and barely visibly detectable. With protein the spectra definitely increases in the purple region.

UV-VIS-NIR Spectrum
CDB Solid Protein - 2nd Generation



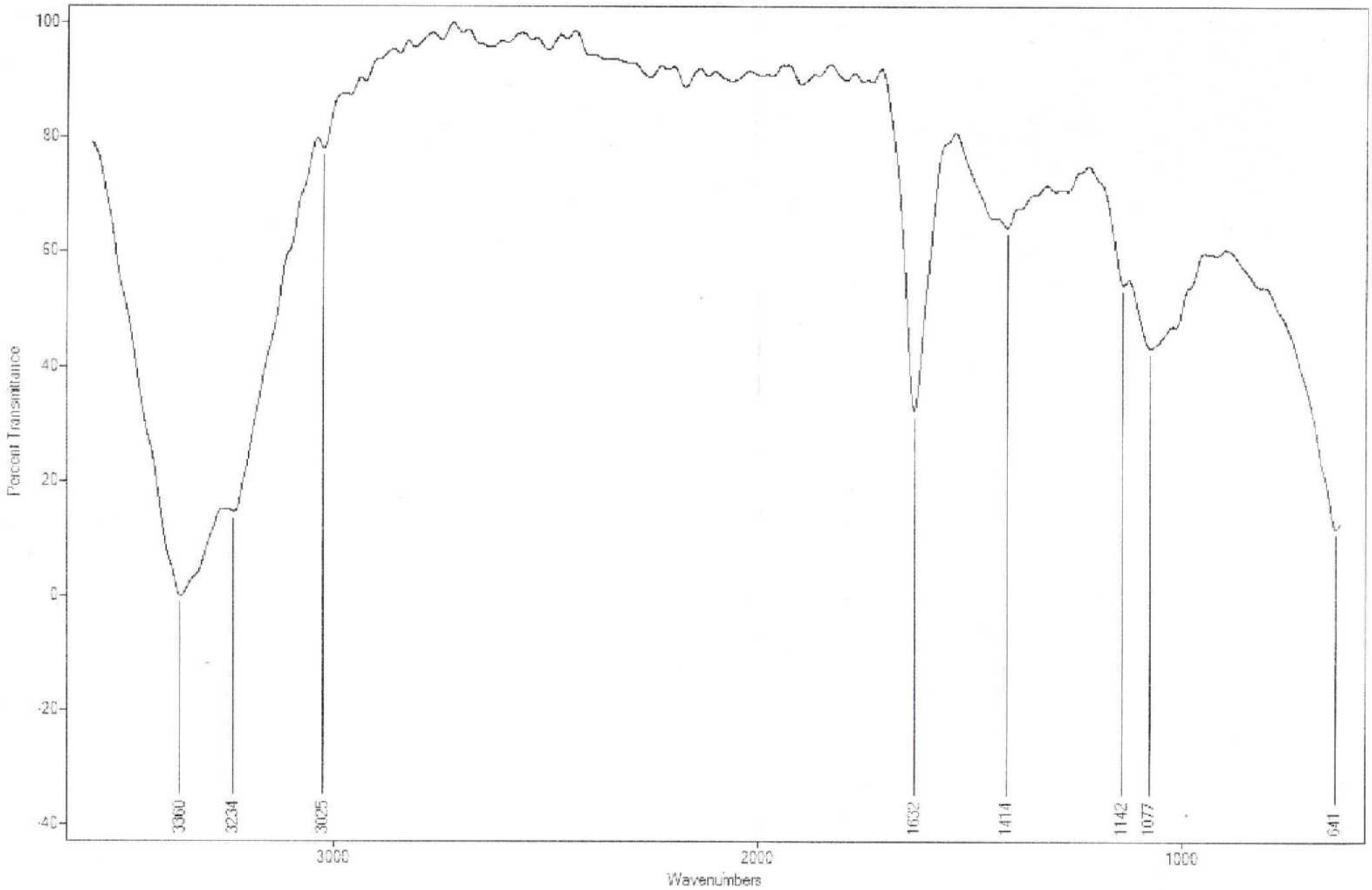


CDB Solid Culture IR Analysis
First Trial Set - Liquid Chromatography
May 26 2019 - Important Spectra Here



CDE Solid Protein - LC - Tube 1-1 - May 25 2019 - 05.spc: Synthetic spectrum by averaging. See audit trail for details.

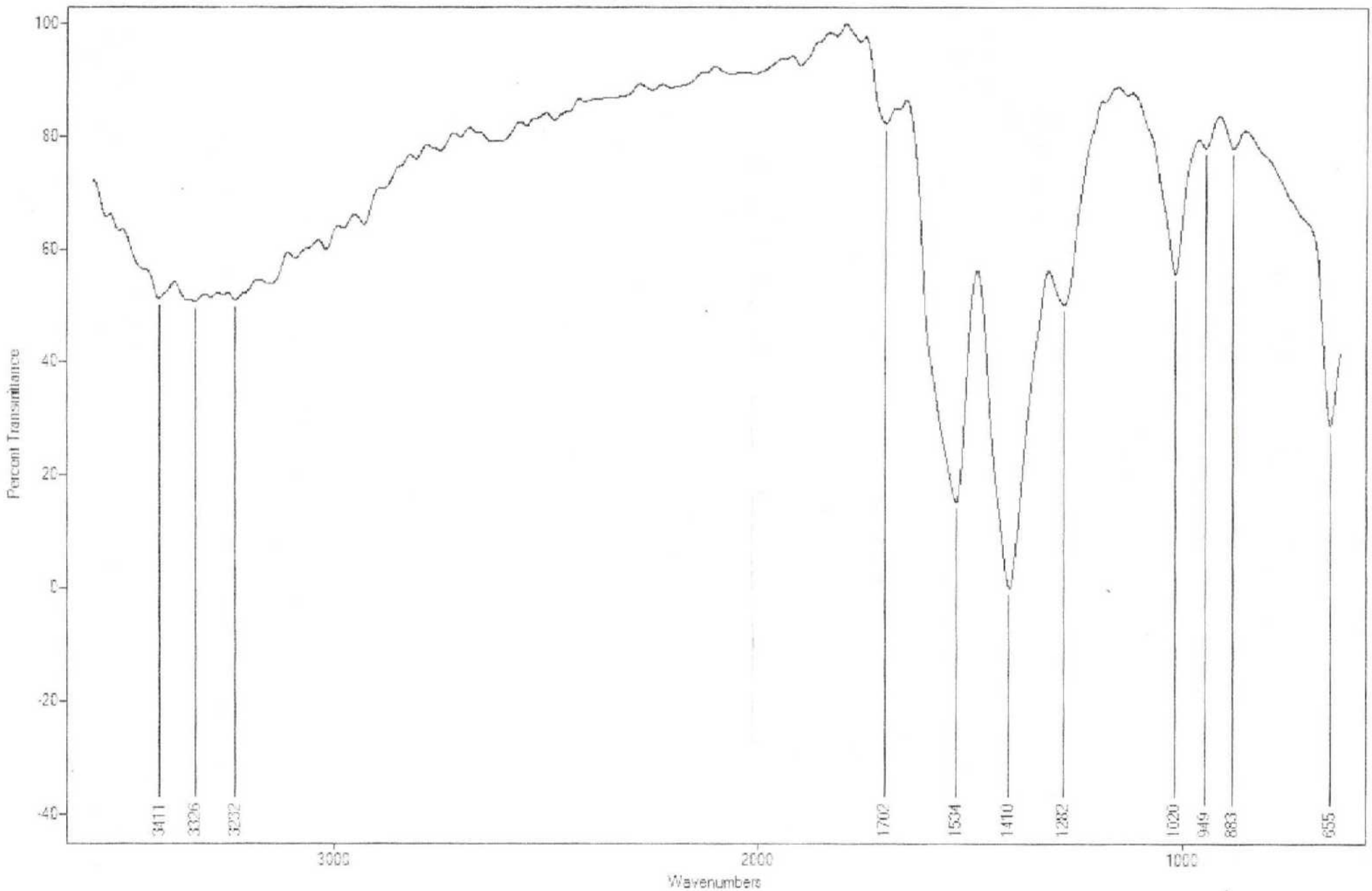
Solvent is H_2O - Cloudy Solution



printed on 05/26/2019 02:53:56

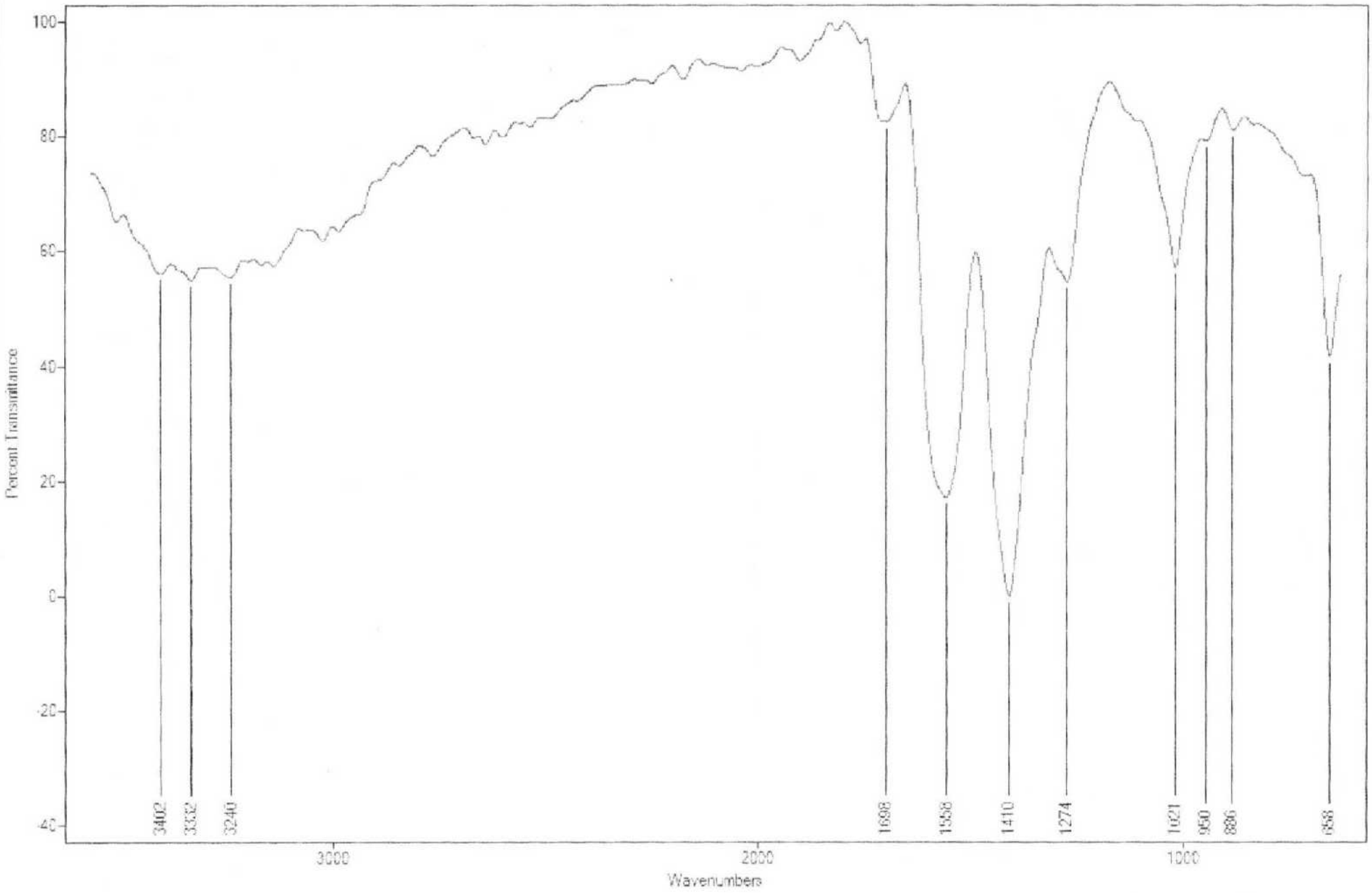
CDS Solid Protein - LC - Tube 1-2 - May 28 2019 - 06.spc: 05/28/2019 10:52:27 title

Solvent is acetic acid - Definite Film Layer



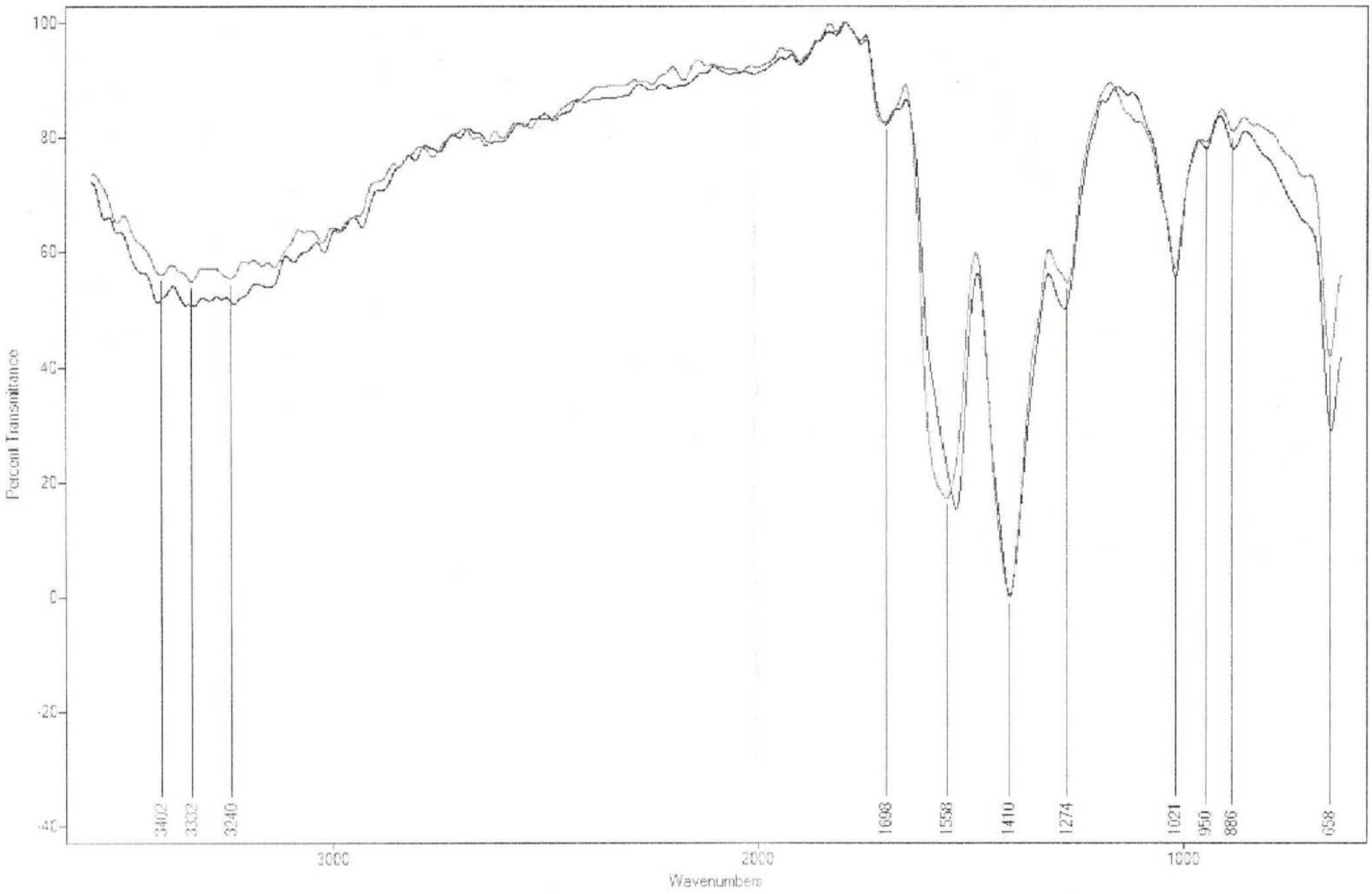
printed on 05/28/2019 11:48:49

CDB Solid Protein - LC - Tube 1-3 - May 26 2019 - 07.spc: 05/26/2019 11:42:47 title



printed on 05/26/2019 11:46:49

CDB Solid Protein - LC - Tube 1-3 - May 28 2019 - 07.spc: 05/28/2019 11:42:47 title

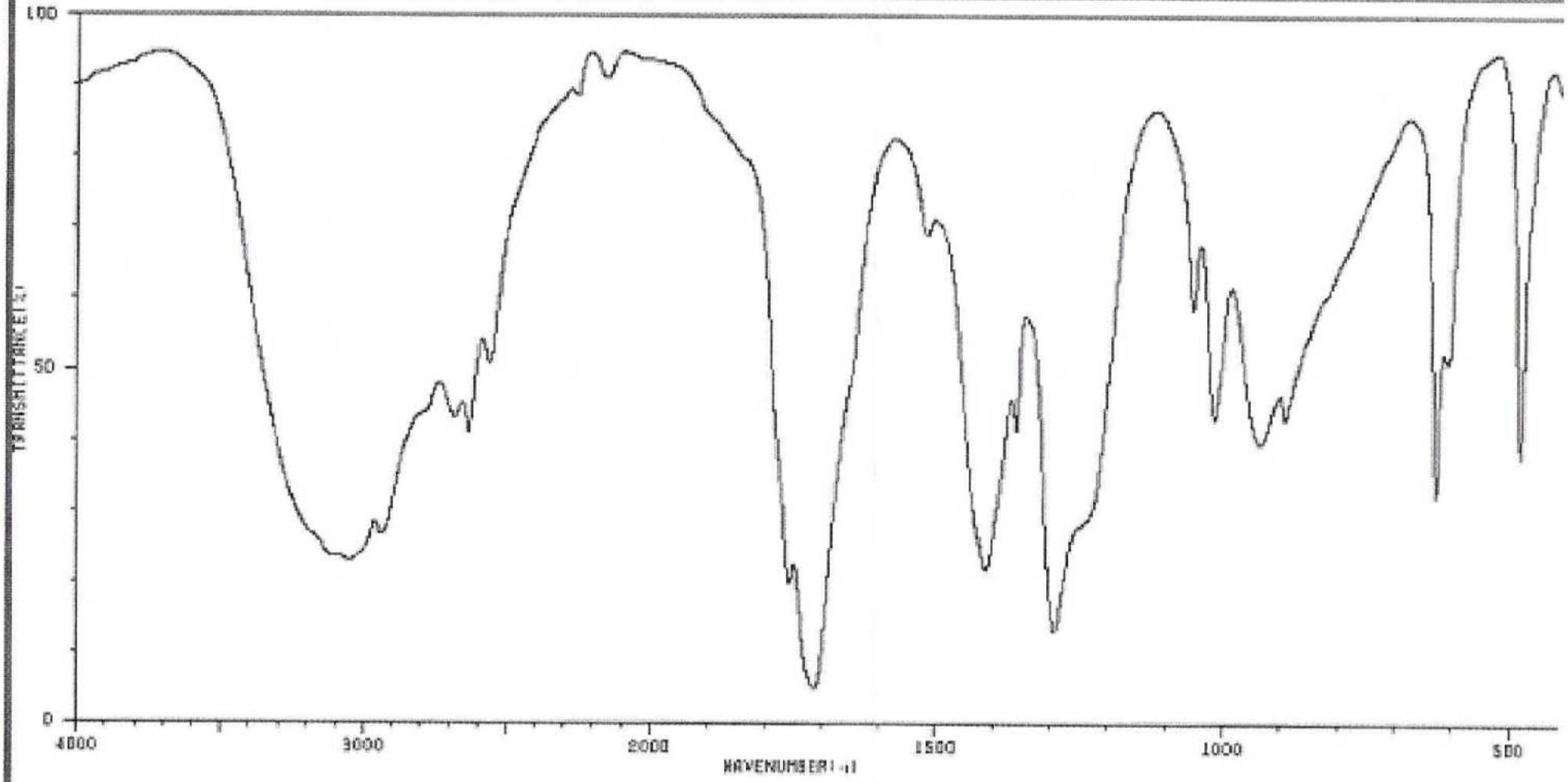


printed on 05/28/2019 11:47:28

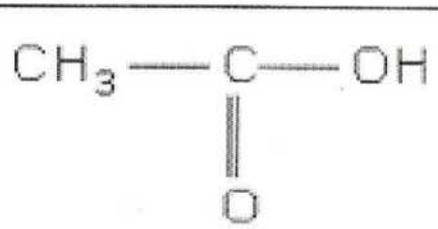
HIT-NO=950 | SCORE= ( ) | SDBS-NO=306 | IR-NIDA-60820 : LIQUID FILM

ACETIC ACID

$C_2H_4O_2$

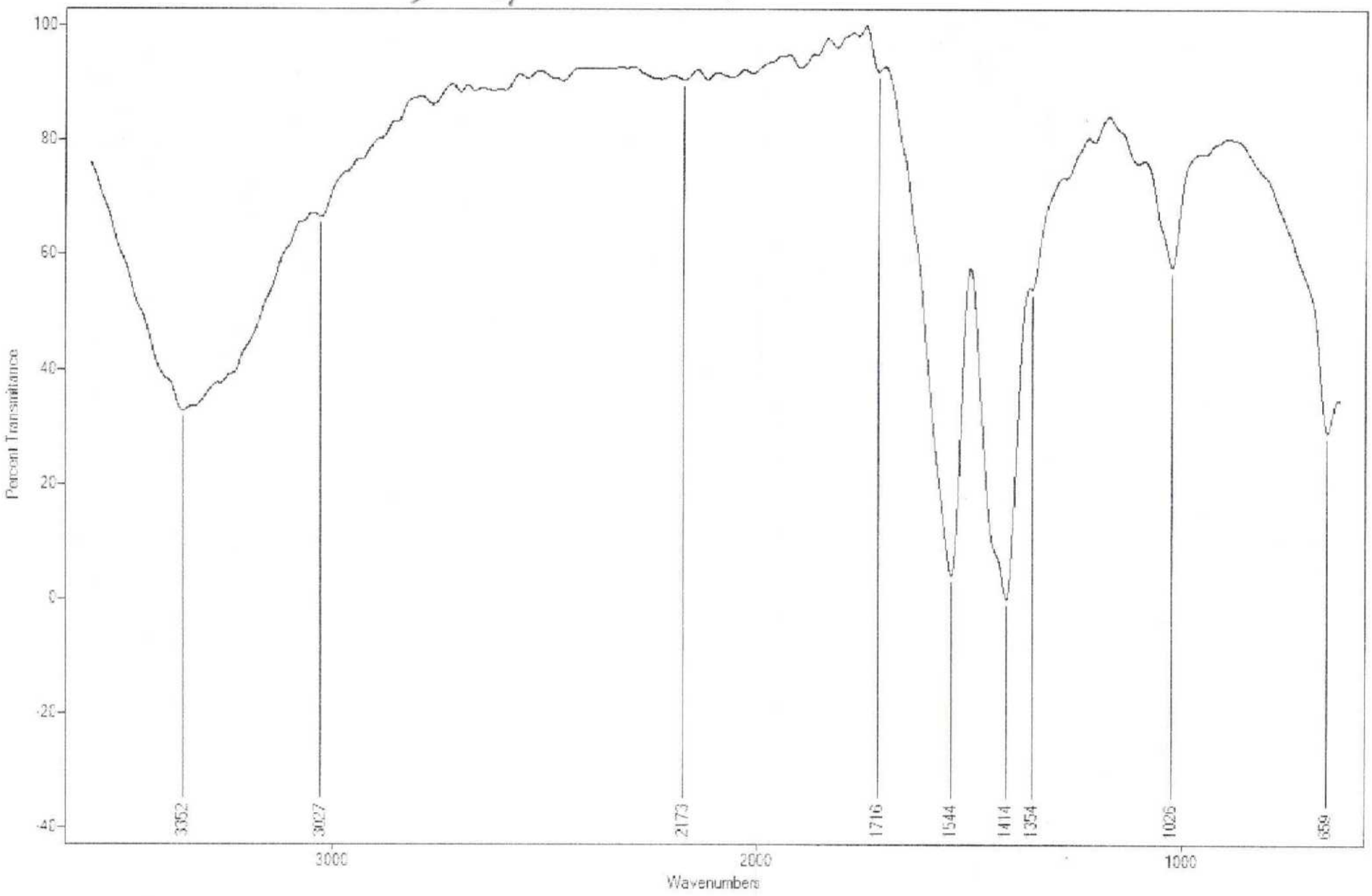


2937	26	1414	20	629	31
2684	41	1360	39	607	49
2631	39	1284	12	481	36
2669	49	1063	67	473	62
1758	19	1018	41		
1714	4	938	57		
1617	66	892	41		



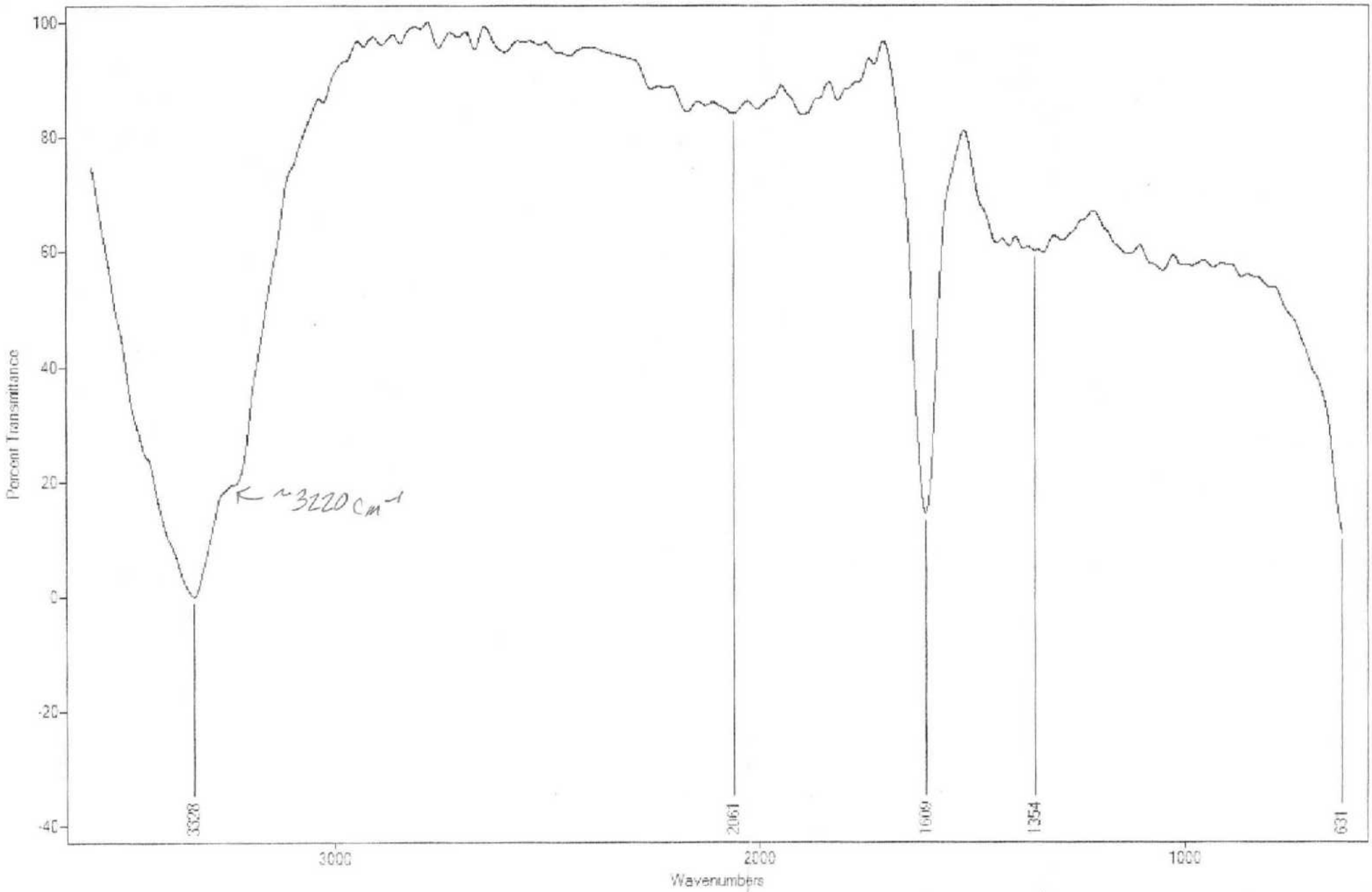
CDB Solid Protein - LC - Tube 1-5 - May 26 2019 - 06.spc: 05/26/2019 15:34:38 title

Solvent is HCl (moderately strong) - Definite Film Layer



printed on 05/26/2019 15:38:00

CD3 Solid Protein - LC - Tube 1-8 - May 26 2019 - 09.spc: 05/26/2019 17:16:45 title



printed on 05/26/2019 17:20:00

OK, the extracted HCl protein, bright yellow in color, definitely passes the test for the existence of Fe^{+3} in the solution.

The test is made w/ Sodium Thiocyanate, and the red Color appears with 1 drop of the HCl protein extract in ~ 3 ml of H_2O.

We know we have Fe^{+3} (Iron in oxidized state)
We know we have NH_2 (proteins)

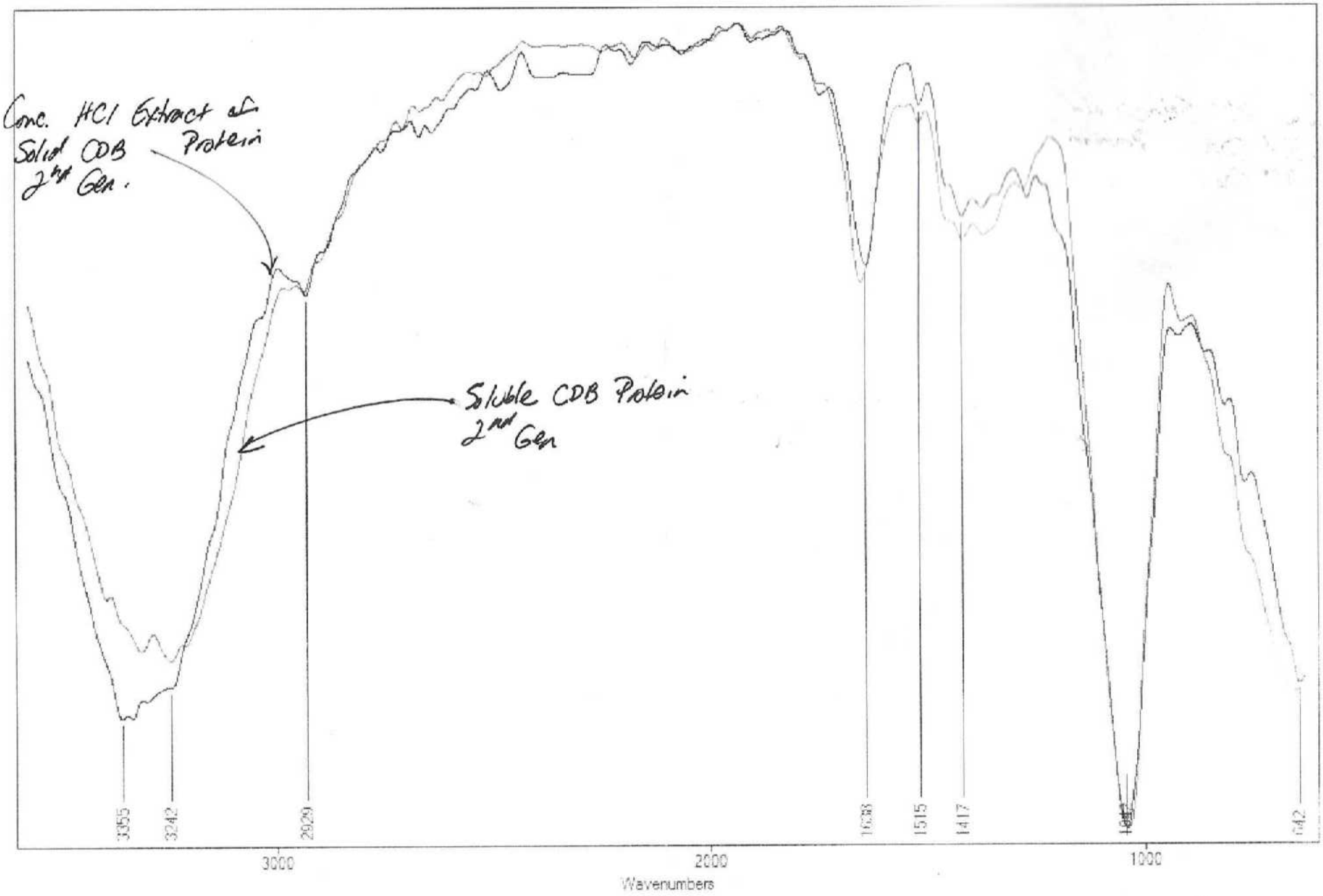
We have now just learned something extremely important. That is that the Concentrated HCl extraction from the solid CDB protein is essentially equivalent to that of the soluble form of the protein.

This means that we expect a level of equivalency of any other analysis methods, such as Liquid Chromatography, Gas Chromatography Pyrolysis results (Carbon - high toxicity level) and IR. We already have equivalency for IR.

We also have the same result when testing for Fe^{+3}. This also says the HCl extracted protein will pass the protein Colorimetric test. We already have protein identified through the functional group NH_2 identification w/ NIR.

We expect the yellow Color to turn brown via oxidation.

CDB Solid Protein 2nd Gen - HCl Extract - Neutral pH - ATR May 25 2019 - 03.spc: Synthetic spectrum by averaging. See audit trail for details.



At this time, the columns are being flushed through w/ water. All sensors appear stable

Notes	t	Conductivity	pH	ORP	Brix	Solvent
Flushing	0040	0.24	9.24	148	0.0	H_2O

Last lesson is that you must neutralize the extract! Strong HCl will destroy the columns. Neutralize to tan color There is some significant precipitation that took place - alkaline

Tube 1-1 IR	0105	3.60	7.98	-20	4.0	H_2O
1-2	0140	5.15	9.02	-2	0.2	H_2O

Tube 1-2 is a much weaker version of Tube 1-1
Tube 1-1 does have an usable IR spectrum

1-3	0210	0.09	9.24	+55	0.1	H_2O

Not Recorded all signs are clear.

Flow Increase 1-4 Isoprop Odor	0245	.02	9.30	+56	22.8	Isoprop

Brix of 90% Isopropanol is 27.6 . Tube Emulsified.
Slight purple color from previous dye run
Tube 1-4 exists as supernatant. No Signal

1-5	0310	.01	0.15	+70	18.9	Ethanol

Ethanol Brix is 9.2
Tubes 1-4 & 1-5 are solvent (alcohol) only. Discarded
Tube 1-2 is too weak - discarded

* 1-2 Film Layer	0930	10.59	5.31	150	5.7	Vinegar

Vinegar is damaging the columns somewhat.
Vinegar Brix is 2.7

* 1-3	1000	7.42	4.91	193	4.0	Vinegar

Tubes 1-2 & 1-3 are the same thing and they are significant. A thin transparent film is produced on the ATR plate. Amide bands seem likely to be present.

Notes	t	Conductivity	pH	ORP	Brix	Solvent
1-4 *	1050	7.32	4.85	195	3.9	Vinegar

There is no reason to think that tube 1-4 is any different than 1-3. 1-2, 3, 4 should all be similar but tube 1-2 is the strongest signal of the set. It is also partly cloudy.

1-5 *	1330	3.75	5.09	226	1.0	Mod HCl
Film layer		HCl solution in reservoir is Brix Ø.3 You have a signal.				
1-6	1410	5.36	6.32	124	1.Ø	Strong HCl

It actually looks like the precipitated material is descending the column, let's keep it going. It has dissolved. Not analyzed yet w/ IR

1-7	1440	4.23	6.48	109	Ø.2	Moderate Strong HCl

As I somewhat surmised, adding strong base to Tube 1-7 created a white precipitate, not sure why yet or what it is, but a degenerate reaction that is sensitive to pH

1-8	1530	9.11	6.64	93	1.3	Strong HCl

Tube 1-8 continues to form a precipitate. Some of the purple dye is being picked up. The column is holding up well!

I have strong UV absorbance @ ~270 nm w/ the white precipitate upon alkaline addition. I believe that it most certainly is protein. Precipitation is regulated by pH.

Notes	t	Conductivity	pH	ORP	Brix	Solvent
1-9	1625	7.97	6.72	95	0.9	ACN

No precipitate being formed any longer. I believe w/ Tube 1-8 I have identified the problem w/ the thioglycanates. Both by IR absorption ~~and by a reaction with acidified protein precipitate and the Fe+3 ion. (ferric chloride solution)~~. NOT JUSTIFIED → There is color produced in the tube (more yellow but nevertheless stronger than the Fe+3 control alone.

There is still some yellow in the column but I cannot get it to elute yet.

I think that our LC separation process is stable @ this time. I do still see some yellow in the mid column, however, I need to pause on the issue we already have four significant separations from the boiled culture material, and each one of them appears to be of a protein.

Ammonia after an encouraging start run is almost immediately separating and removing residual ~~blue~~ purple dye from the column. The yellow residual protein remains mid column.

Your idea of repairing acid damage to the sand column (has worked) extremely well. I have added a cut off inverted pipette to a heating tube/bulb and created a vacuum pump to suck and withdraw the gravel cap on the top. Replacement of lost sand and adding a new gravel cap has been perfectly executed. About 1-1½ of the sand column had been damaged/lost.

In addition, the acid strength used here was somewhat unusual due to the nature of the protein set, only being soluble in highly concentrated acid. Alkaline pH precipitates the protein in all cases.

—

Additional runs after Tube 1-9 are w/ ethanol (no obvious result) and w/ ammonia (cleared residual purple dye from previous runs).

—

The separated proteins can be re-precipitated w/ the addition of a base, but not the HCl dissolved protein. Thus for the precipitated protein (i.e. Tubes 1-6 thru 1-8 produce an absolutely soluble (presumed) protein.

May 21 2019

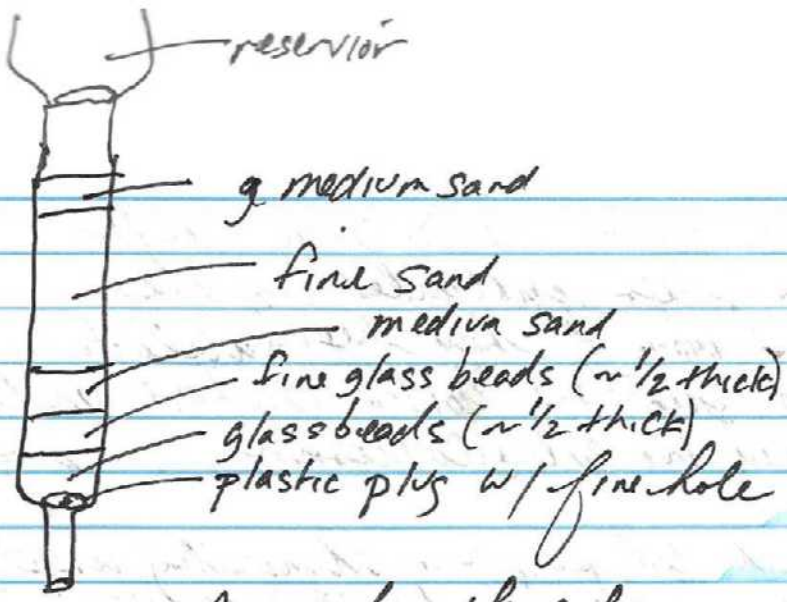
The column is plugged today. Trying a variety of solvents to see if it can be opened up. No luck so far.
Currently acetone, Vinegar, HCl combination.

Based upon the success of yesterday (approx 15 large tube separations) column is not worth the time waiting for. Repack the column

Ok, a very good decision to repack the column. No combination of solvents would never have solved that problem.

The problem lies in the design of the specific new (smaller) column being used. They chose to insert a fine hole plastic washer type plug immediately before the outlet. It was plugged up and it is very hard to get to. I do not need or want the plug, but it cannot be removed. I was eventually able to clear the plug and have now redesigned the bottom cap of the column. The goal is to never have any non soluble reach the outlet of the column - difficult to ensure.

Now we have:



all together we have about a 1 1/2" end cap now!

I now have the column working again.

Now, the actual source of the problem lies in that a portion of the protein (a solid culture growth, not necessarily attracted to protein) remains insoluble in the presence of concentrated HCl. However, HCl does go a long way in dissolving the proteins (solids) and for that I am grateful.

Now, after filtering the dissolved solvent, the introduction of different bases will precipitate various solids. If done w/in the column, such as with KOH or Ammonia, it has the very real potential to plug the column and it did. It is difficult to avoid the situation but we must try.

The best solution is that any solids that precipitate as a result of alkaline solvent we must be removed immediately or as soon as possible to avoid working themselves deeper into the column where it can be plugged.

If the particular fritted plug washer had not been installed in the column I would have been able to eject it from the bottom without undoing & repacking the entire column.

Overall, it did perform admirably until ~~the precipitation~~ I attempted to dissolve excessive precipitation w/ Conc. HCl. This is a delicate balance

—

To construct a column in the field, you will now need:

1. glass beads (~ 2-3 mm)
2. fine glass beads (~ 1 mm)
3. Coarse sand
4. fine sand
5. Specific diameter cleaning wire for plug.

—

Today the goal is to attempt a repeat of the separation sequence remarkably achieved yesterday (w/ IR plots of all significance)

and to attempt further verification of protein existence via UV absorbance & Colorimetric testing.

And try to keep the column flowing.

First step in column sample prep is to neutralize the concentrated HCl dissolved culture solids, but not to the point of precipitation.

Or another method is if they do precipitate remove those solids from the column.

The reason for this is that the strong HCl forms very hard on the column. Back off on neutralization slightly before the precipitation starts to occur.

I have started a repeat column run. I have the sample still in acid state today in an attempt to avoid some of the precipitation problem, at least for the take home. There will be fructy. The column flow is extremely slow right now during the settling of the sample, but it is not plugged.

For intermediate work, let us conduct UV and colorimetric analyses on the separations from yesterday to establish any protein prospects.

After sample settling in, the columns are now flowing normally w/ H_2O as the first solvent.

Red Dye Must Be Mixed Fresh
200ul in 12ml H_2O

Let's get our protein colorimetric test notes recalled from Vol 17 Notes p.204, May 22 2017

3ml H_2O
~~20ul RT red dye~~ (diluted dye 1/2 of 1/2 H_2O)
~~6 drops 1M NaOH~~ 1 drop 10M NaOH
1 drop 0.5M $CuSO_4$
pinch tartaric acid

→ Use 200 ul dye in 12 ml H_2O

MUST BE MIXED FRESH!!

Color shift is to purple.
Test dye w/ powdered milk
532 nm appear to be test wavelength

Let's look the red dye activation
20ul 1/2 & 1/2 means

$\frac{10ul}{3ml\ H_2O} = \frac{100ul}{30ml\ H_2O}$

Then a way too strong.

Ok, we have some modifications here.

First off, the dye strength should be mixed @ a level of 500ul per 30ml H_2O

The reagent recipe is now

1. Mix ~~500 ul red dye (RIT~~ Cherry Red) in 30 ml H_2O
   ~~Call the the diluted dye~~
   ~~200 ul in 12 ml H_2O~~
   Now: Red Dye MUST BE FRESH!

1. 3 ml H_2O (no more than this!)
2. 100 ul diluted dye
3. ~~6 drops 1M NaOH~~ 1 drop 10M NaOH
4. 2 drops (TWO!) 0.5M $CuSO_4$
5. Pinch Tartaric acid (sufficient to eliminate precipitate)

Now add protein:
Control is blue (no protein)
Shifts to purple (with protein)

Also, mix the dye using a pipette & 30 ml H_2O separately – do not contaminate the micro-pipette!

The is a fairly sensitive test, quite sensitive actually. It is able to detect ~ 5 grains of powdered milk w/in the 3 ml sample.

The purple shift will develop more fully if additional time can be allotted.
~668, 512 nm Control maximums – VIS
~640, 533 nm Shift Purple w/ Protein.

* 512 to 533 indicates a shift from red towards purple
668 to 640 is a shift from blue green toward greenish blue

LC Trial #2

Notes	t	Conductivity	pH	ORP	Brix	Solvent
2-1	1515	> 19.99	7.85	-59	2.0	H_2O

Tube 2-1 does not pass the colorimeter test for protein.

Actually, this is not entirely true. With sufficient time, there may have been a slight shift towards purple. UV, VIS, IR all can be helpful here. A difficult call, it has clouded the reagent solution some but not necessarily shifted to purple

We see that Tube 2-1 matches Tube 1-1 by IR. They are the same component and the repeats of the May 26 LC separations.

Let's get a quick read on IR

3354 cm^{-1} NH amine or amide indicated

1634 cm^{-1} Carbonyl indicated (1600-1820)

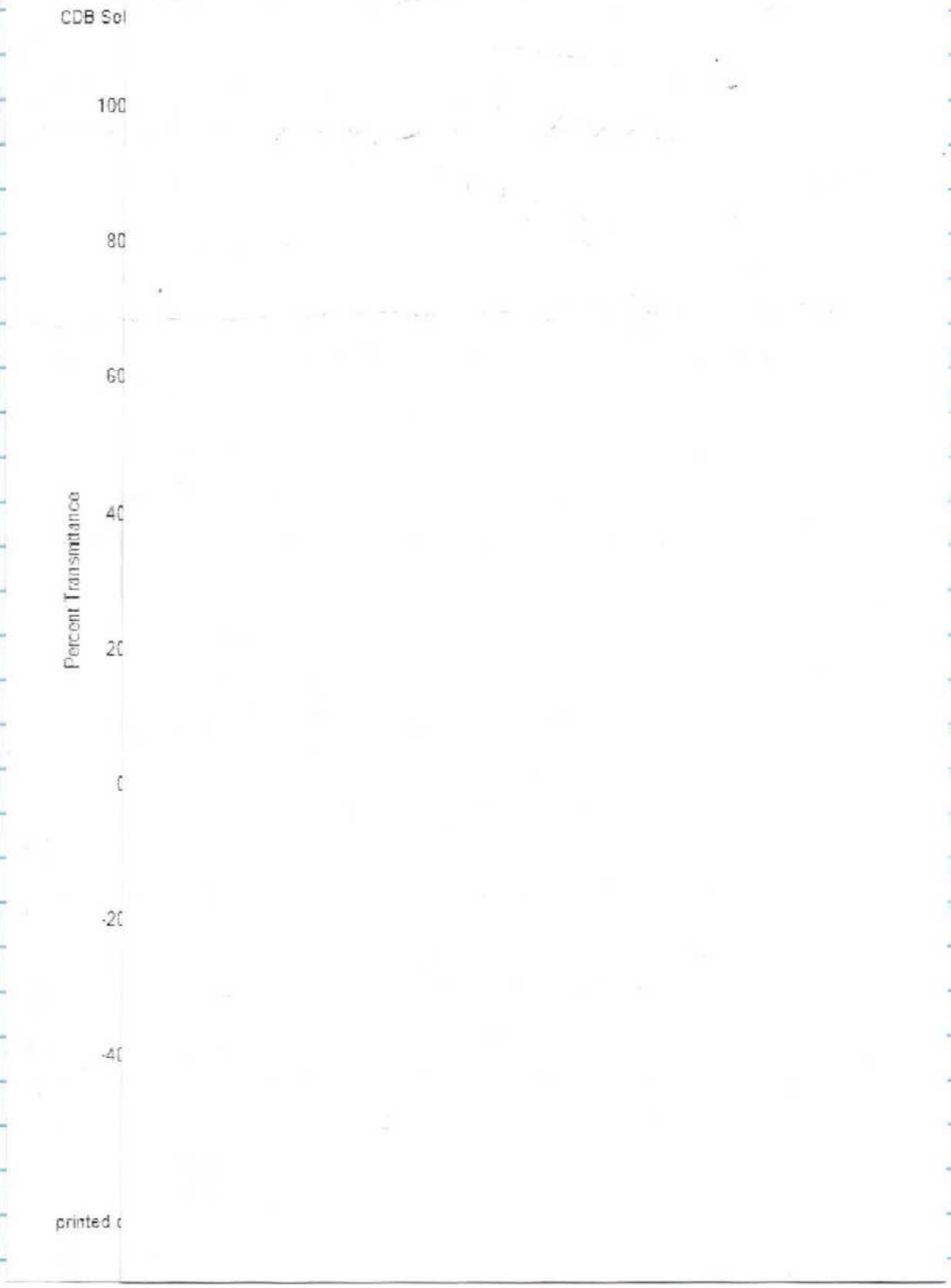
1408 cm^{-1} C-N Aliphatic amine, Sulfonic Ester

1270 weak

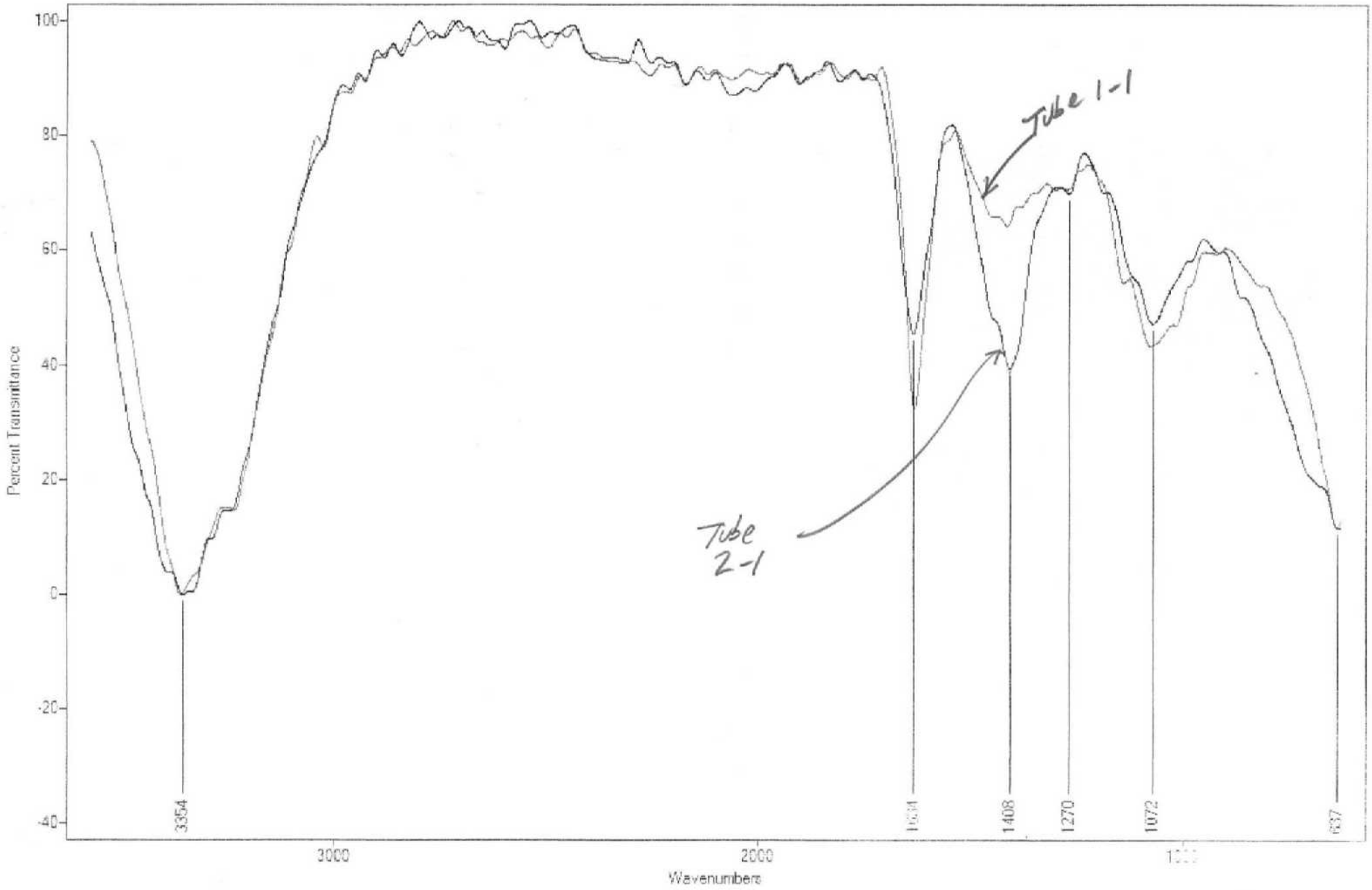
1012 cm^{-1} Esters (1300-1000)

There are no good matches of Tube 2-1 w/ the master library/database so we have a new compound of some type.

Tube 2-1 IR LC



CDB Solid Protein - LC - Tube 2-1 - May 28 2019 - 10.spc: Synthetic spectrum by averaging. See audit trail for details.



printed on 05/27/2019 16:52:26

Notes	#	Conductivity	pH	ORP	Brix	Solvent
2-2	1605	4.01	8.58	+58	0.2	H_2O

Tube 2-2 is essentially the same as Tube 2-1 except notice that we pick up an additional peak @ 3235 cm^{-1}

An amine salt is a possibility here

~3280 NH_3^+ amine salt in solution

I am performing a ninhydrin test for a amine on both 2-1 & 2-2. Both Tubes 2-1 & 2-2 fail the ninhydrin amine test. So now we look for an ester.

this is what i found

The only straightforward reaction
alcohol.

One method is to dissolve the sus
drops of KOH solution and a drop
be pink (alkaline colour of pheno

Now place the test tube and conte
tube containg all the reagents bu
indicator colour fades, comparing
will hydrolyse and the acid produ
to its colourless, acid form.

Tube 2-2 is essentially the same as Tube 2-1 except notice that we pick up an additional peak @ 3235 cm^{-1}

An amine salt is a positively charged

~3280 NH_3^+ amine salt in solution

I am performing a ninhydrin test for a amine on both 2-1 & 2-2. Both Tubes 2-1 & 2-2 fail the ninhydrin amine test. So now we look for an ester.

Untitled

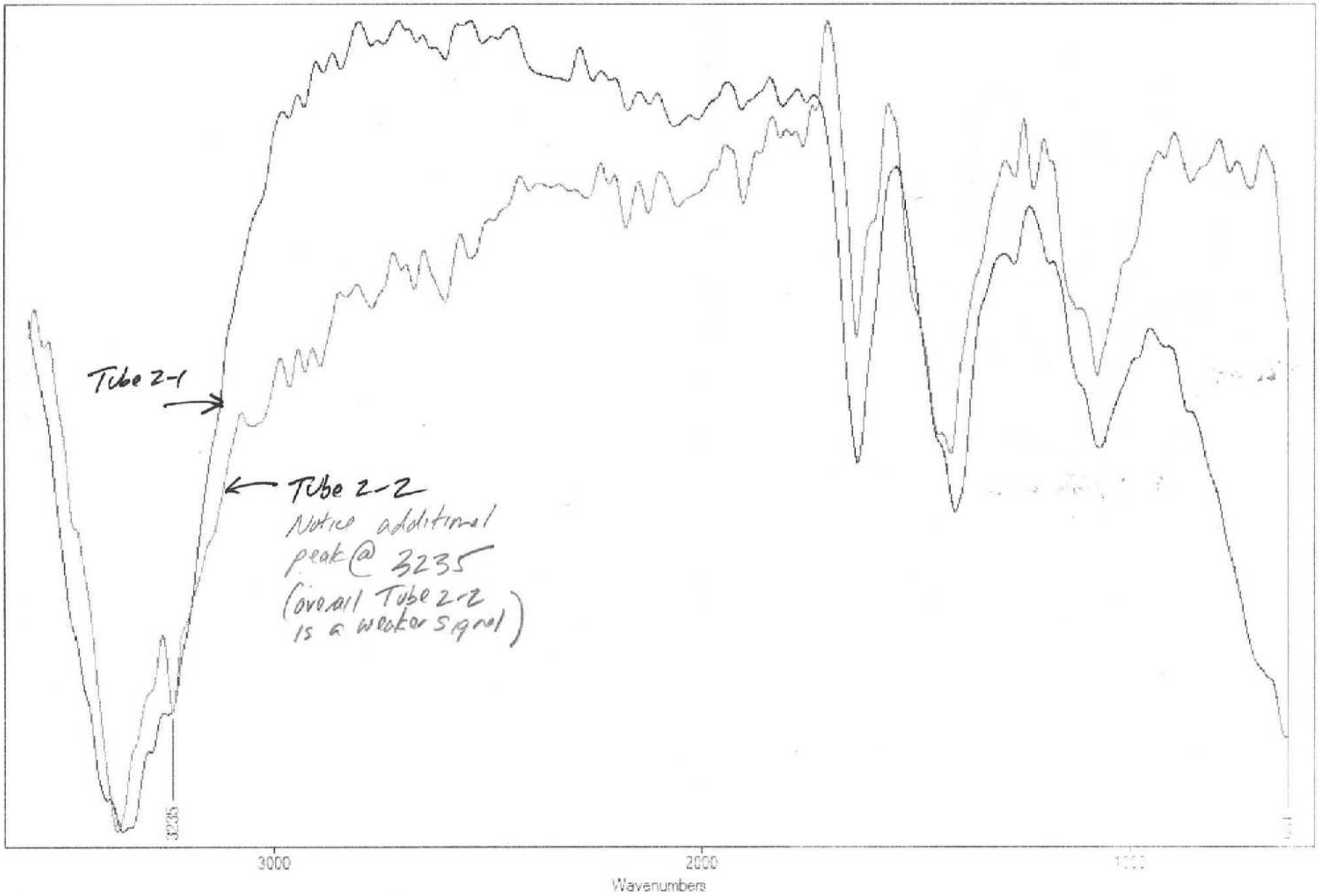
this is what i found

The only straightforward reaction of esters is hydrolysis to their original acid and alcohol.

One method is to dissolve the suspected ester in a little ethanol and then add a few drops of KOH solution and a drop or two of phenolphthalein. The solution should now be pink (alkaline colour of phenolphthalein).

Now place the test tube and contents in a boiling water bath together with another tube containg all the reagents but not the suspected ester. See whether the indicator colour fades, comparing it with the 'blank'. The point is that the ester will hydrolyse and the acid produced will neutralise the KOH and turn the indicator to its colourless, acid form.

CDB Solid Protein - LC - Tube 2-2 - May 26 2019 - 11.spc: 05/27/2019 17:45:16 title



printed on 05/27/2019 17:47:43

Looking @ our notes, we see that the compound separated from the g water only become weaker and weaker, which is what we already see.

Next we see that alcohols had no effect upon separation, either w/ isopropanol or ethanol.

The change to vinegar as our next solvent. Vinegar is now active as the solvent.

With respect to the test for esters, @ the point a negative test is indicated.

However, we notice that in the KOH treated tube that we definitely have a precipitate that has formed under alkaline conditions. They was under a condition of heating. Tube 2-2 should serve as a useful secondary test sample to work w/ on the question

	t	EC	pH	ORP	Brix	Solvent
2-3 Two Tubes	1615	5.81	4.74	252	2.6	Vinegar

Yes, we do have a purple color shift WITH TUBE 2-2!!!
It is weak, but it is visible.

I actually thought that I was testing Tube 2-3 but that is not the case. I was testing tube 2-2, which by IR is almost identical to Tube 2-1, but Tube 2-1 is more cloudy. We also have a very clean white precipitate forming in Tube 2-2 under alkaline & heat conditions. Tube 2-2 has a very low protein concentration but it passes the colorimetric test.

Retract

~~OC - on Tube 2-3 I positively have a protein and it is fairly strong. I also have lots of sample available since I have 2 full tubes of separation. This is super.~~

~~Two separations, two proteins.~~

Retraction with respect to (w.r.t) Tube 2-3.
Tube 2-3 has an acidic pH. The acidic state is what produced a color change, not protein. This was suspected because the color change was to reddish purple, not purple.

I need to carefully review and examine the circumstances of Tube 2-3.

Tube 2-3 has a pH of ~ 4.5, ie fairly acidic. I am fairly sure I let the column run too long with too much vinegar on the sample. I am thinking that you will need to cancel the run @ this time.

The acid nature of Tube 2-3 is significantly distorting the results of the protein colorimetric test. Turning the reagent acidic changes its color to red to red purple.

That is not the purple dye that comes from protein existence.

When the pH changes dramatically you are distorting the results of the protein colorimetric test!

An additional IR analysis of Tube 2-3 should continue to confirm the problem. Let us see.

OK Confirmed by IR. This LC run is to be scrapped. I allowed too much vinegar to flow through the column.

Disregard all assessments regarding Tube 2-3. Assessments on Tube 2-1 & 2-2 remain tentative.

Now, fortunately we still have the LC run of May 26 2019 (yesterday) available to make assessments on.

Let us conduct Colorimetric Protein tests on these separations. Recall that a PURPLE shift is required to substantiate the existence of protein.

NO PROTEIN

1. Protein Colorimetric test: Tube 1-1:
   Result is NEGATIVE

SAME PROTEIN HERE

2. Protein Colorimetric test: Tube 1-2:
   Result is definite POSITIVE
   It did require 400 ul of Tube 1-2 however, in the 3 ml of H_2O to reflect the purple shift. 500 ul gave a very strong result

PROTEIN HERE

3. Protein Colorimetric Test: Tube 1-3 Stronger Concentration
   Result is definite Positive
   200 ul+ required to produce purple shift.
   1-2, 3, 4 are estimated to be the same.

4. Protein Colorimetric Test 5.09
   Tube 1-5 Notice pH here is ~~3.65~~ - Caution.

Protein Here (Most Likely)

Possible marginal result here.
However >1200+ ul indicate marginal concentration of at all. Also caution advised from low pH influences.
With passage of time, test result does appear positive

5. Protein Colorimetric test Tube 1-6
pH is 6.32 - this is most favorable

Result is negative.

6. Protein Colorimetric test: Tube 1-8
Result is negative (pH 6.64)

The result of the protein Colorimetric test is as follows:

1. Two proteinaceous separations are indicated:

a) Tube (1-2, 1-3)
b) Tube 1-5

2. Two non proteinaceous compounds are indicated (ie IR signal also acquired)

a) Tube 1-1
b) Tube 1-8 (Recall that a precipitate forms here w/ addition of alkaline)

These are our testing spots for today.

On tap is:

1. Run all Trial 1 series through UV for additional assessment
2. Analyze IR plots & compare to colorimetric protein test results.
3. Recall Amene and retest that negative results
4. Get a repeat LC set in agreement w/ Trial #1.

May 20 2019

Since trial #2 has been scrapped, I will repeat the LC trial today as #3. The column is working superbly. You must remember to neutralize the concentrated HCl extract as quickly as possible w/en the column or it will damage the column.

	t	EC	pH	ORP	Brix	Solvent
3-1	1235	720	7.12	-20	3.8	H_2O

Notice upon repeat now, that the first eluate is extremely conductive. The acetate interpretation of composition. Notice also the cloudiness of solution repeats and that a precipitate of some form does settle out over time.

Upon settlery of the column, we see that we have a definite settlery out of a precipitate.

Any examination going forward will need to distinguish between the two states collected in the separation, one liquid and the other a precipitate.

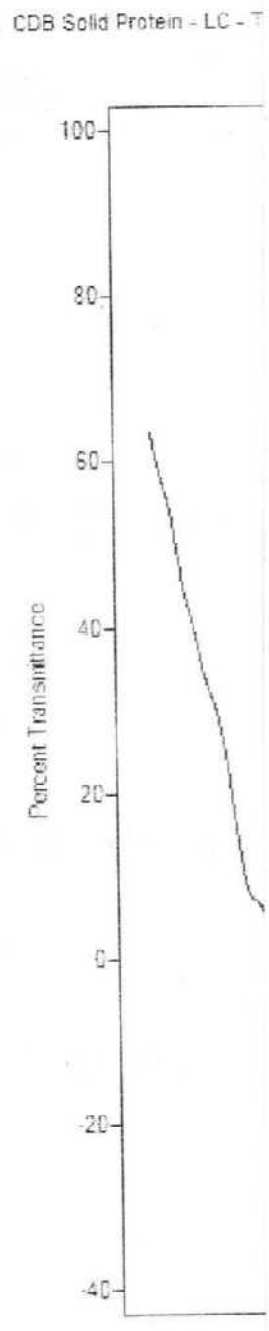
Let's start w/ the liquid separation.

IR Peaks:
3351, 3242
1634
1142, 1275 wok
1062, 646

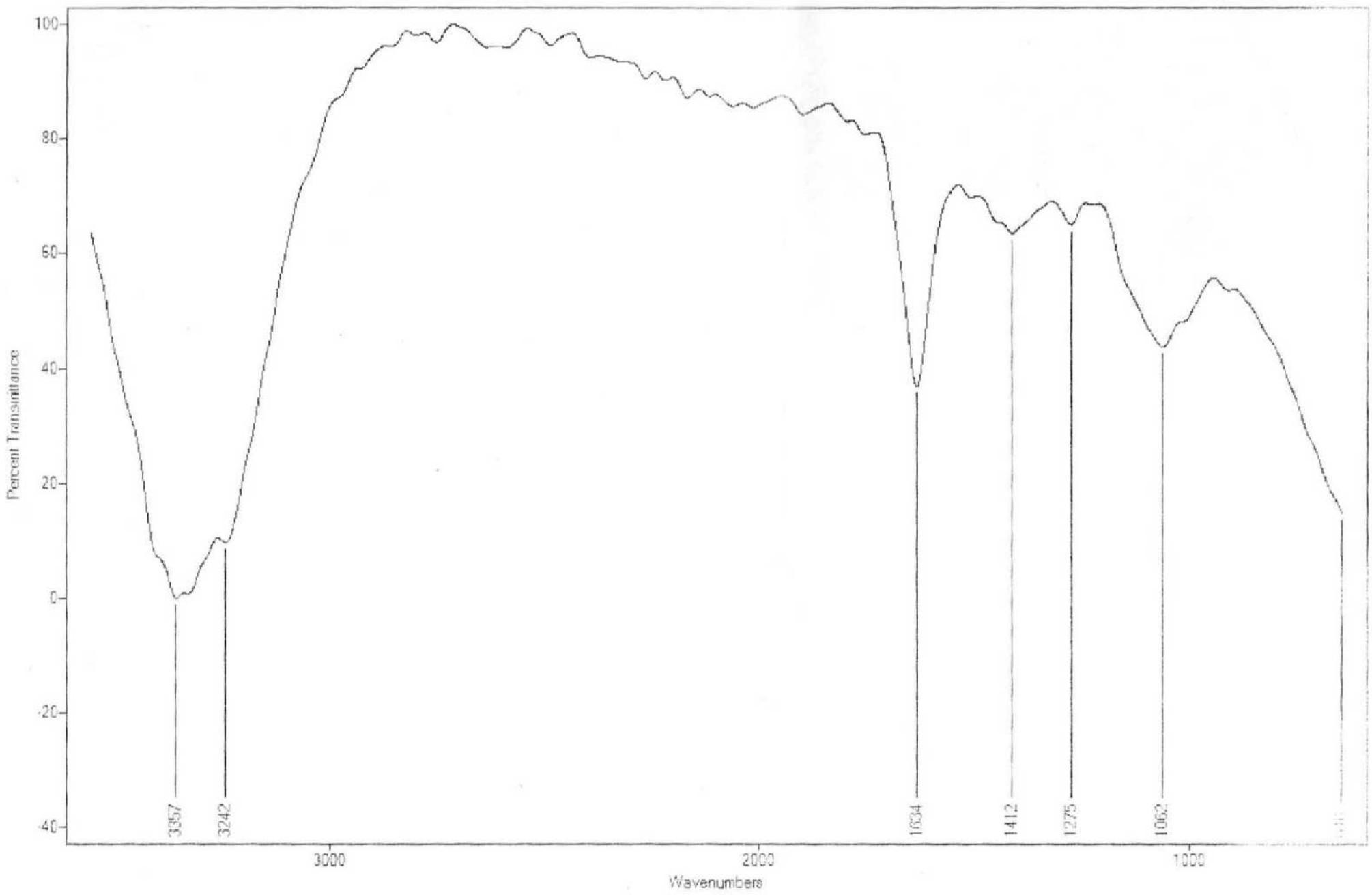
IR Plot here in Tube 3-1 - Solution ONLY

Repeatable Compound, high conductivity, Precipitate handled separately

	t	EC	pH	ORP	Brix	Solvent
3-2	1335	3.12	8.29	+90	Ø.3	H_2O



CDB Solid Protein - LC - Tube 3-1 - SOLUTION ONLY - May 28 2019 - 12.spc: 05/28/2019 14:56:37 title



FTIR

printed on 05/28/2019 15:25:48

Next, let's look @ Tube 3-1, Solution only with UV and the Colorometric protein test.

We have absorbance with UV @ ~ 273nm. This strongly suggests protein.
Let's look @ powdered milk as a reference.

With powdered milk as a control reference we notice that the UV absorbance maximum is @ ~ 279nm - definitely closer to the 280nm conventional point.

Question: Can different protein types shift the reference slightly?

From several sources, the 280nm value is an approximate value. As much

as 10-20nm variability in absorption is referenced therefore we are well in that range (~ 7nm).

The presumption of protein in solution in Tube 3-1 is well founded, however very low concentration is surmised.

A control solution is always being used in the protein colorometric test, ie, one tube has no protein in it.

Tube 3-1 Protein Colorimetric Test:
At this point, the results of this test are NEGATIVE. There may be an issue of concentration more than any other.

When completing the colorimetric test you must also be very mindful of the potential influence of pH on the results. Remember that the state of the reagent MUST remain alkaline @ the conclusion of the test. The pH of each sample should be recorded and kept in mind during each test.

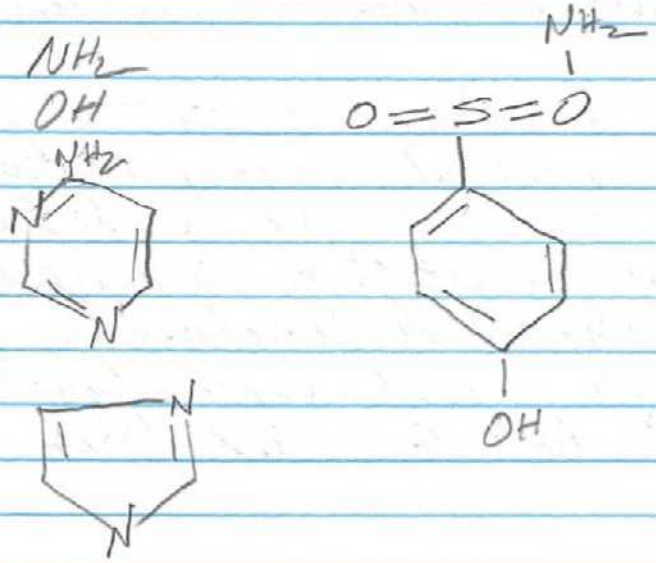
The pH of Tube 3-1 is 7.12 so it should have no significant acidic influence.

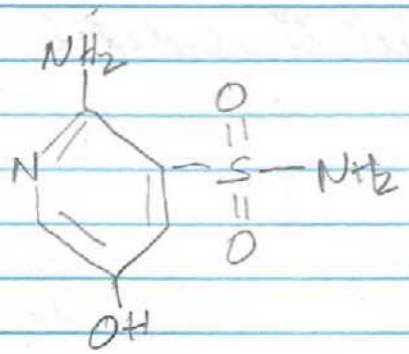
The IR spectrum will be needed to finish the scale you are to problem beforehand. Time to also start using SDBS database.

Using the entire set of absorbance values (< 70% T, +/- 10 cm^{-1}) SDBS shows only three candidates for a match. Two are reasonable.

1. 5-Hydroxy, 1-Naphthalene sulfonamide
2. 2-Aminoadenosine

Let us now look @ the maximum functional group set that encompasses the two candidate compounds found.





This would be a reasonable proposed compound, or somethg w/ these attributes.

We know that our compound is highly polar.

Sulfonamide
Aminoadenosine
} categories

SDBS IR Database - Japan
Candidate Compounds for Tube 3-1
Solution Only

SDBS No:	34938	**CAS Registry No.:**	2096-10-8
DOI:			
Molecular Formula:	$C_{10}H_{14}N_6O_4$	**Molecular Weight:**	282.3

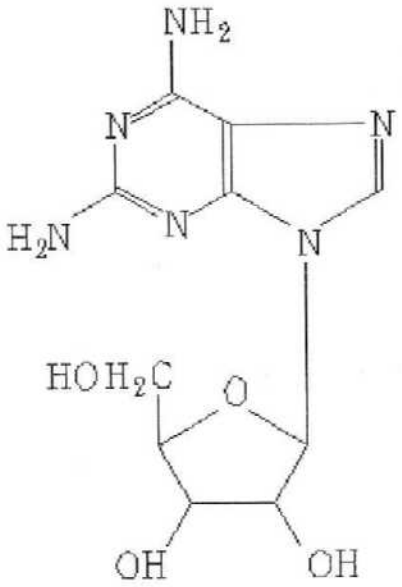
SDBS-NO= 34938
2-AMINOADENOSINE

SDBS No: 34938 **CAS Registry No.:** 2096-10-8

DOI:

Molecular Formula: $C_{10}H_{14}N_6O_4$ **Molecular Weight:** 282.3

SDBS-NO= 34938
2-AMINOADENOSINE



Peaks used
3537 cm⁻¹
3242
1634
1062
646
T < 60% ±10 nm

Compound Name:

2-aminoadenosine
2,6-diamino-9-(beta-D-ribofuranosyl)purine
2,6-diaminopurine riboside
9-(beta-D-ribofuranosyl)-9H-purine-2,6-diamine
adenosine, 2-amino-
adenosine-2-amine
purine-2,6-diamine ribonucleoside
purine-2,6-diamine riboside

InChI:

InChI=1S/C10H14N6O4/c11-7-4-8(15-10(12)14-7)16(2-13-4)9-6(19)5(18)3(1-17)20-9/h2-3,5-6,9,17-19H,1H2,(H4,11,12,14,15)/t3-,5-,6-,9-/m1/s1

InChIKey:

ZDTFMPXQUSBYRL-UUOKFMHZSA-N

Publisher:

National Institute of Advanced Industrial Science and Technology (AIST)

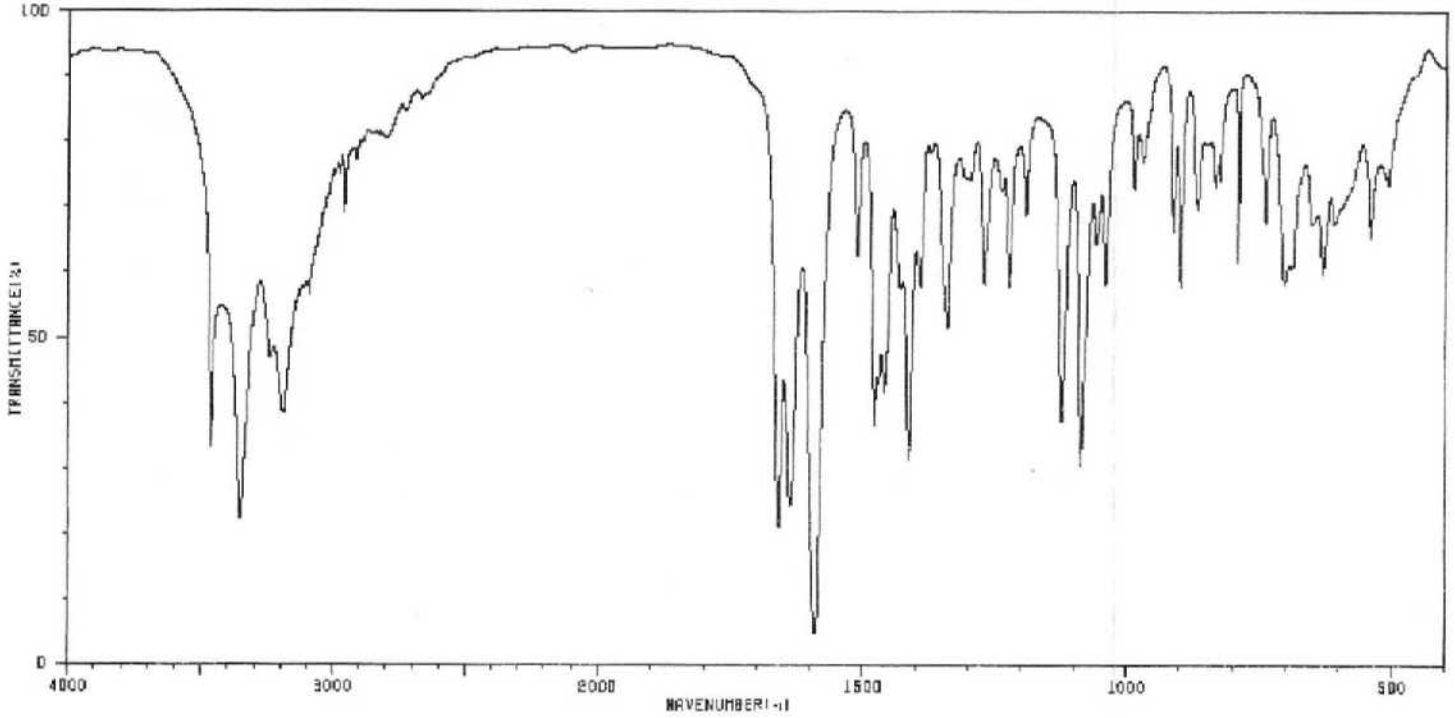
Rights:

Date of Publication:

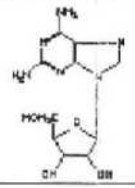
1999-03-31

Update History:

HIT-NO=20225	SCORE= ( )	SDBS-NO=34938	IR-NIDA-54608 ; KBR DISC
2-AMINOADENOSINE			
$C_{10}H_{14}N_6O_4$			



3464	32	1638	28	1384	55	1058	62	691	68
3353	21	1591	4	1342	49	1041	55	653	64
3240	44	1512	60	1272	55	914	64	635	57
3190	37	1480	36	1225	55	900	66	611	64
3094	55	1472	41	1198	68	791	58	602	68
2961	66	1460	58	1124	35	740	66	594	68
1860	20	1416	30	1087	29	706	66	542	62



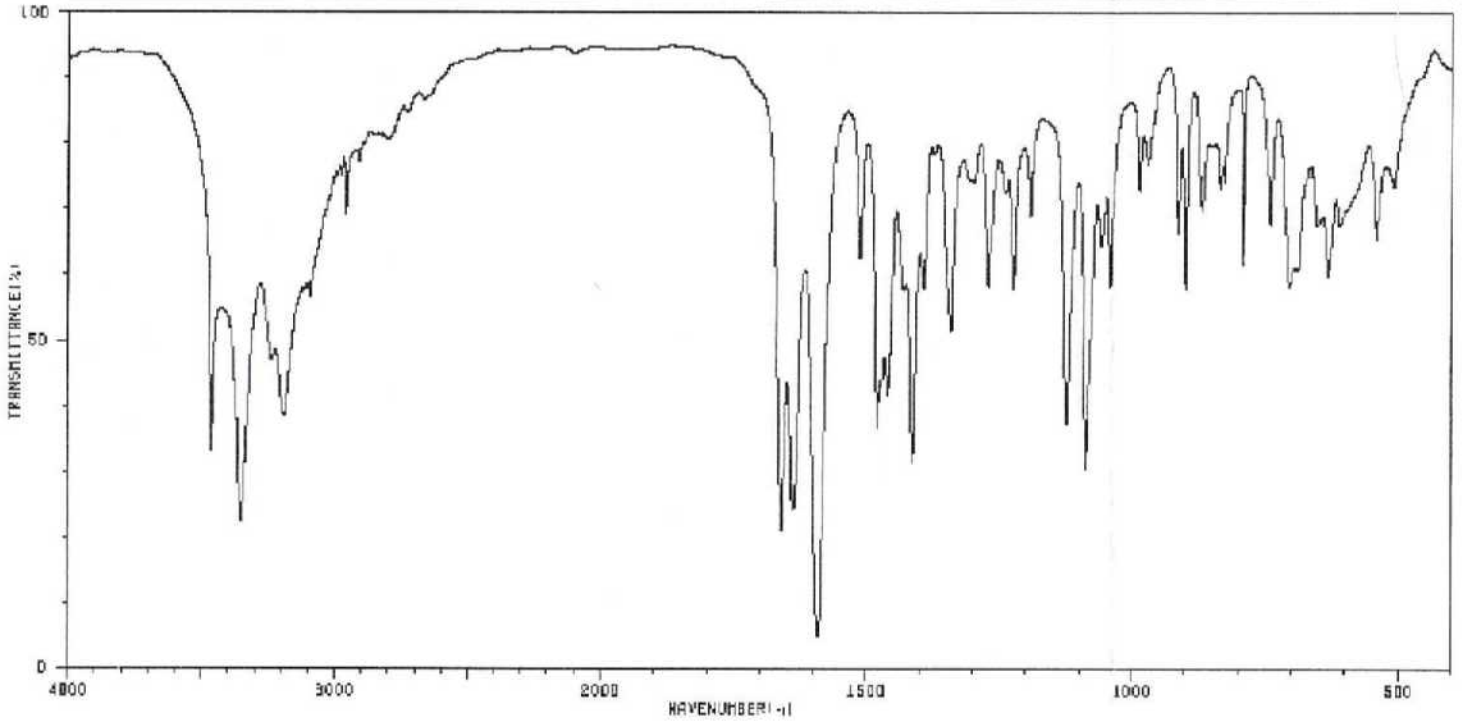
All peaks used
T < 70%
+/- 10nm

Also subset of strong peaks used:

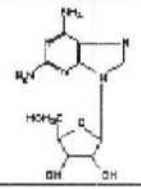
3531 cm^{-1}
3242
1634
1062
646

T ≤ 60%
+/- 10nm

HIT-NO=20225	SCORE= ( )	SDBS-NO=34938	IR-NIDA-54608 : KBR DISC
2-AMINOADENOSINE			
$C_{10}H_{14}N_6O_4$			



3464	32	1638	25	1394	55	1059	62	691	58
3353	21	1591	4	1342	49	1041	55	653	54
3240	44	1512	60	1272	55	914	64	636	57
3190	37	1480	36	1225	55	900	55	611	64
3094	55	1472	41	1198	58	791	58	602	58
2961	66	1460	38	1124	35	740	66	594	58
1660	20	1416	30	1087	29	706	55	542	62



2 Hits

Full Peak list

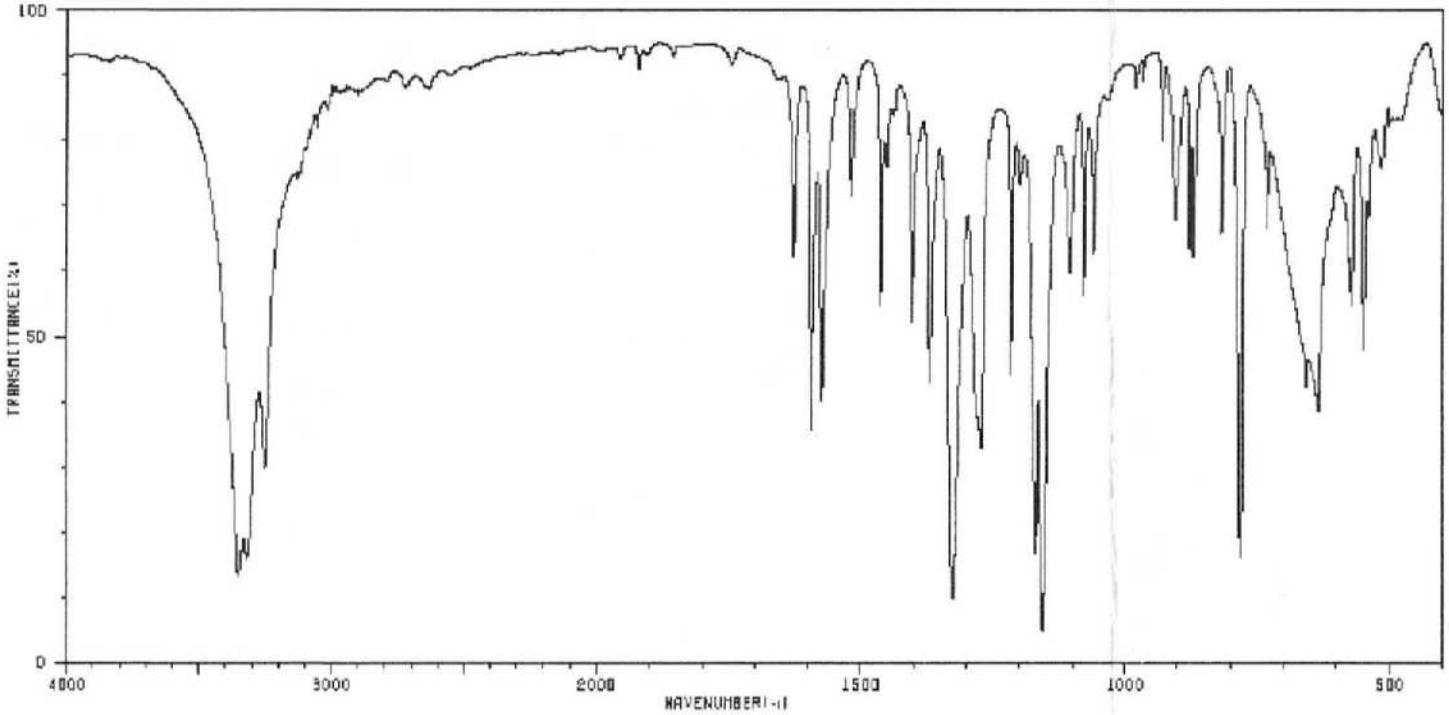
Strong subset peak list

HIT-NO=9950	SCORE= ( )	SDBS-NO=23135	IR-NIDA-34717 : KBR DISC

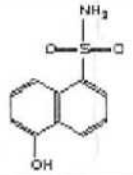
5-HYDROXY-1-NAPHTHALENESULFONAMIDE

$C_{10}H_9NO_3S$

All peaks used
T < 70%
+/- 10nm



3350	12	1518	69	1216	42	929	77	657	41
3319	15	1463	52	1200	70	904	66	634	37
3249	26	1452	72	1171	16	877	60	579	56
3062	79	1403	60	1158	4	869	60	572	52
1627	60	1370	41	1106	57	817	64	549	46
1594	34	1328	9	1079	63	761	15	540	66
1573	38	1273	31	1060	60	731	64	516	72



SDBS Information

SDBS No.: 23135

Compound Name:
5-hydroxy-1-naphthalenesulfonamide

Molecular Formula: $C_{10}H_9NO_3S$

Molecular Weight: 223.3

CAS Registry No.:
17286-26-9

Spectral Code:
Mass :
^{13}C NMR : in DMSO-d_6
^{1}H NMR : 400 MHz in 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=23135

External Information:
external link displays in a separate page

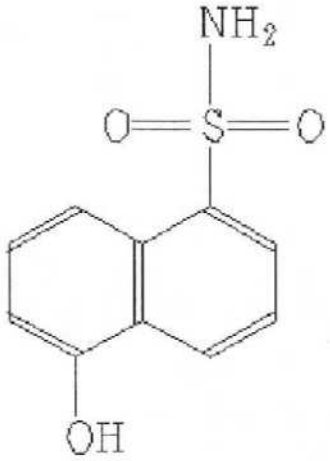
- Japan Chemical Information Link Center (in Japanese)
  - JST Nikkaji Web (in Japanese)

SDBS No: 23135 **CAS Registry No.**

DOI:

Molecular Formula: $C_{10}H_9NO_3S$ **Molecular Weigl**

SDBS-NO= 23135
5-HYDROXY-1-NAPHTHALENESULFONAMIDE



Compound Name:
5-hydroxy-1-naphthalenesulfonamide

InChI:
InChI=1S/C10H9NO3S
/c11-15(13,14)10-6-2-3-7-8(10)4-1-5-9(7)12/h1-6,12H,
(H2,11,13,14)

InChIKey:
NFVBVKHGDDDCEA-UHFFFAOYSA-N

Publisher:
National Institute of Advanced Industrial Science and Technology (AIST)

Rights:
AIST all rights reserved

Date of Publication:
1999-03-31

Update History:

WIKIPEDIA

Sulfonamide (medicine)

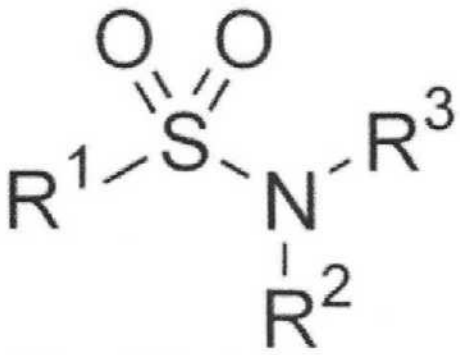
Sulfonamide is a functional group (a part of a molecule) that is the

Sulfonamide (medicine) - Wikipedia
Discussions

WIKIPEDIA

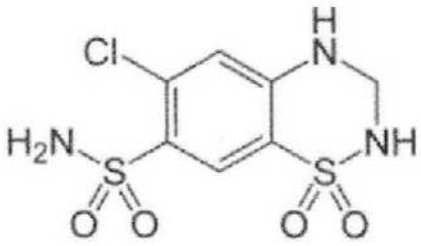
Sulfonamide (medicine)

Sulfonamide is a functional group (a part of a molecule) that is the basis of several groups of drugs, which are called **sulphonamides**, **sulfa drugs** or **sulpha drugs**. The original antibacterial sulfonamides are synthetic (nonantibiotic) antimicrobial agents that contain the sulfonamide group. Some sulfonamides are also devoid of antibacterial activity, e.g., the anticonvulsant sultiame. The sulfonylureas and thiazide diuretics are newer drug groups based upon the antibacterial sulfonamides.[1][2]



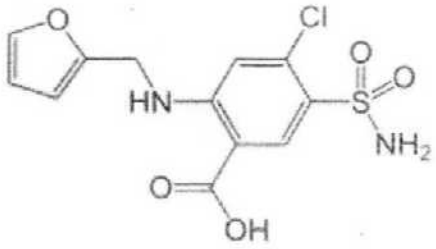
Sulfonamide functional group

Allergies to sulfonamides are common. The overall incidence of adverse drug reactions to sulfa antibiotics is approximately 3%, close to penicillin;[3] hence medications containing sulfonamides are prescribed carefully. It is important to make a distinction between sulfa drugs and other sulfur-containing drugs and additives, such as sulfates and sulfites, which are chemically unrelated to the sulfonamide group, and do not cause the same hypersensitivity reactions seen in the sulfonamides.



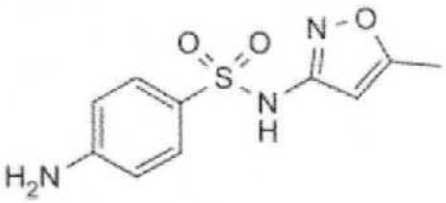
Hydrochlorothiazide is a sulfonamide and a thiazide.

Nowadays, while sulfonamides seldom appear in the prescriptions written by doctors in developed countries, sulfonamides are still common antimicrobial medications in developing countries owing to their low price.[4]:414–416[5]:337–343



Furosemide is a sulfonamide, but not a thiazide.

Contents



Sulfamethoxazole is an antibacterial sulfonamide

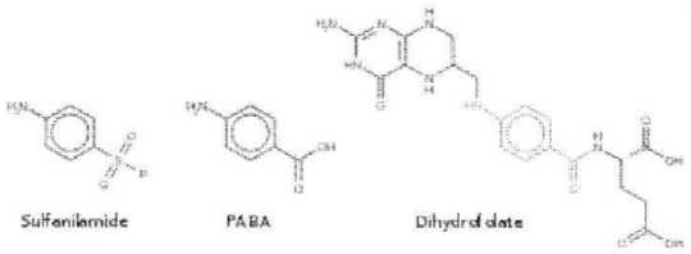
Function

Antimicrobial

In bacteria, antibacterial sulfonamides act as competitive inhibitors of the enzyme dihydropteroate synthase (DHPS), an enzyme involved in folate synthesis. Sulfonamides are therefore bacteriostatic and inhibit growth and multiplication of bacteria, but do not kill them. Humans, in contrast to bacteria, acquire folate (vitamin B_9) through the diet.[6]

Other uses

Sulfonamides are used to treat allergies and cough, as well as antifungal and antimalarial functions. The moiety is also present in other medications that are not antimicrobials, including thiazide diuretics (including hydrochlorothiazide, metolazone, and indapamide, among others), loop diuretics (including furosemide, bumetanide, and torsemide), acetazolamide, sulfonylureas (including glipizide, glyburide, among others), and some COX-2 inhibitors (e.g., celecoxib).



Structural similarity between sulfonamide (left) and PABA (center) is the basis for the inhibitory activity of sulfa drugs on tetrahydrofolate (right) biosynthesis

Sulfasalazine, in addition to its use as an antibiotic, is also used in the treatment of inflammatory bowel disease.[7]

History

Sulfonamide drugs were the first antibacterials to be used systemically, and paved the way for the antibiotic revolution in medicine. The first sulfonamide, trade-named Prontosil, was a prodrug. Experiments with Prontosil began in 1932 in the laboratories of Bayer AG, at that time a component of the huge German chemical trust IG Farben. The Bayer team believed that coal-tar dyes which are able to bind preferentially to bacteria and parasites might be used to attack harmful organisms in the body. After years of fruitless trial-and-error work on hundreds of dyes, a team led by physician/researcher Gerhard Domagk[8] (working under the general direction of Farben executive Heinrich Hörlein) finally found one that worked: a red dye synthesized by Bayer chemist Josef Klarer

that had remarkable effects on stopping some bacterial infections in mice.[9] The first official communication about the breakthrough discovery was not published until 1935, more than two years after the drug was patented by Klarer and his research partner Fritz Mietzsch.

Prontosil, as Bayer named the new drug, was the first medicine ever discovered that could effectively treat a range of bacterial infections inside the body. It had a strong protective action against infections caused by streptococci, including blood infections, childbed fever, and erysipelas, and a lesser effect on infections caused by other cocci. However, it had no effect at all in the test tube, exerting its antibacterial action only in live animals. Later, it was discovered by Bovet,[10] Federico Nitti and J. and Th. Jacques Tréfouël, a French research team led by Ernest Fourneau at the Pasteur Institute, that the drug was metabolized into two pieces inside the body, releasing from the inactive dye portion a smaller, colorless, active compound called sulfanilamide.[11] The discovery helped establish the concept of "bioactivation" and dashed the German corporation's dreams of enormous profit; the active molecule sulfanilamide (or sulfa) had first been synthesized in 1906 and was widely used in the dye-making industry; its patent had since expired and the drug was available to anyone.[12]

The result was a sulfa craze.[13] For several years in the late 1930s, hundreds of manufacturers produced tens of thousands of tons of myriad forms of sulfa. This and nonexistent testing requirements led to the elixir sulfanilamide disaster in the fall of 1937, during which at least 100 people were poisoned with diethylene glycol. This led to the passage of the Federal Food, Drug, and Cosmetic Act in 1938 in the United States. As the first and only effective antibiotic available in the years before penicillin, sulfa drugs continued to thrive through the early years of World War II.[14] They are credited with saving the lives of tens of thousands of patients, including Franklin Delano Roosevelt Jr. (son of US President Franklin Delano Roosevelt) and Winston Churchill.[15][16] Sulfa had a central role in preventing wound infections during the war. American soldiers were issued a first-aid kit containing sulfa pills and powder, and were told to sprinkle it on any open wound.

The sulfanilamide compound is more active in the protonated form. The drug has very low solubility and sometimes can crystallize in the kidneys, due to its first pK_a of around 10. This is a very painful experience, so patients are told to take the medication with copious amounts of water. Newer analogous compounds prevent this complication because they have a lower pK_a, around 5–6, making them more likely to remain in a soluble form.

Many thousands of molecules containing the sulfanilamide structure have been created since its discovery (by one account, over 5,400 permutations by 1945), yielding improved formulations with greater effectiveness and less toxicity. Sulfa drugs are still widely used for conditions such as acne and urinary tract infections, and are receiving renewed interest for the treatment of infections caused by bacteria resistant to other antibiotics.

Preparation

Sulfonamides are prepared by the reaction of a sulfonyl chloride with ammonia or an amine. Certain sulfonamides (sulfadiazine or sulfamethoxazole) are sometimes mixed with the drug trimethoprim, which acts against dihydrofolate reductase. As of 2013, the Republic of Ireland is the largest exporter worldwide of sulfonamides, accounting for approximately 32% of total exports.[17]

List of sulfonamides

Trimethoprim Sulfadiazine Azulfidine (sulfasalazine)

WIKIPEDIA

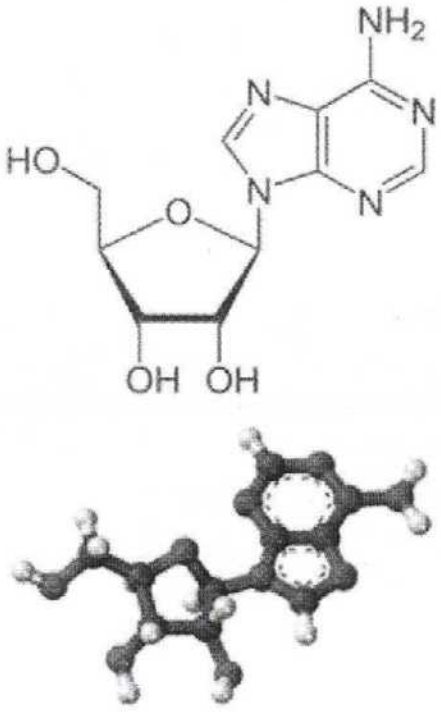
Adenosine

Adenosine is both a chemical found in many living systems and a medication. As a medication it is used to treat certain forms of supraventricular tachycardia that do not improve with vagal maneuvers.[1] Common side effects include chest pain, feeling faint, shortness of breath along with tingling of the senses.[1] Serious side effects include a worsening dysrhythmia and low blood pressure.[1] It appears to be safe in pregnancy.[1]

It is a purine nucleoside composed of a molecule of adenine attached to a ribose sugar molecule (ribofuranose) moiety via a β-N_9-glycosidic bond.[2][3][4] Derivatives of adenosine are widely found in nature and play an important role in biochemical processes, such as energy transfer—as adenosine triphosphate (ATP) and adenosine diphosphate (ADP)—as well as in signal transduction as cyclic adenosine monophosphate (cAMP). Adenosine itself is a neuromodulator, believed to play a role in promoting sleep and suppressing arousal. Adenosine also plays a role in regulation of blood flow to various organs through vasodilation.[5][6][7]

Contents

Adenosine



Clinical data	
Trade names	Adenocard; Adenocor; Adenic; Adenoco; Adeno-Jec; Adenoscan; Adenosin; Adrekar; Krenosin
Synonyms	SR-96225 (developmental code name)
AHFS/Drugs.com	Monograph (https://www.drugs.com/monograph/adenosine.html)
Pregnancy category	C (adenosine may be safe to the fetus in pregnant women)
Routes of administration	Intravenous
ATC code	C01EB10 (WHO (https://www.whocc.no/atc_ddd_index/?code=C01EB10))
Legal status	
Legal status	In general: ℞ (Prescription only)
Pharmacokinetic data	
Bioavailability	Rapidly cleared from circulation via cellular uptake

Protein binding	No
Metabolism	Rapidly converted to inosine and adenosine monophosphate
Elimination half-life	cleared plasma <30 seconds; half-life <10 seconds
Excretion	can leave cell intact or can be degraded to hypoxanthine, xanthine, and ultimately uric acid

Identifiers

IUPAC name	
CAS Number	58-61-7 (http://www.commonchemistry.org/ChemicalDetail.aspx?ref=58-61-7) ✓
PubChem CID	60961 (https://pubchem.ncbi.nlm.nih.gov/compound/60961)
IUPHAR/BPS	2844 (http://www.guidetopharmacology.org/GRAC/LigandDisplayForward?ligandId=2844)
DrugBank	DB00640 (https://www.drugbank.ca/drugs/DB00640) ✓
ChemSpider	54923 (http://www.chemspider.com/Chemical-Structure.54923.html) ✓
UNII	K72T3FS567 (https://fdasis.nlm.nih.gov/srs/srsdirect.jsp?regno=K72T3FS567)
KEGG	C00212 (http://www.kegg.jp/entry/C00212) ✓
ChEBI	CHEBI:16335 (https://www.ebi.ac.uk/chebi/searchId.do?chebiId=CHEBI:16335) ✓
ChEMBL	ChEMBL477 (https://www.ebi.ac.uk/chembldb/index.php/compound/inspect/ChEMBL477) ✗
CompTox Dashboard (EPA)	DTXSID1022558 (https://comptox.epa.gov/dashboard/DTXSID1022558)
ECHA InfoCard	100.000.354 (https://echa.europa.eu/substance-information/-/substanceinfo/100.000.354)

Chemical and physical data

Formula	$C_{10}H_{13}N_5O_4$
Molar mass	267.241 g/mol g·mol^{-1}
3D model (JSmol)	Interactive image (https://chemapps.stolaf.edu/jmol/jmol.php?model=n2c1c%28ncnc1n%28c2%29%5BC%40%40H%5D3O%5BC%40%40H%5D%28%5BC%40%40H%5D%28O%29%5BC%40H%5D3O%29CO%29N)
SMILES	
InChI	

Medical uses

Supraventricular tachycardia

In individuals with supraventricular tachycardia (SVT), adenosine is used to help identify and convert the rhythm.

Certain SVTs can be successfully terminated with adenosine.[8] This includes any re-entrant arrhythmias that require the AV node for the re-entry, e.g., AV reentrant tachycardia (AVRT), AV nodal reentrant tachycardia (AVNRT). In addition, atrial tachycardia can sometimes be terminated with adenosine.

Fast rhythms of the heart that are confined to the atria (e.g., atrial fibrillation, atrial flutter) or ventricles (e.g., monomorphic ventricular tachycardia) and do not involve the AV node as part of the re-entrant circuit are not typically converted by adenosine. However, the ventricular response rate is temporarily slowed with adenosine in such cases.

Because of the effects of adenosine on AV node-dependent SVTs, adenosine is considered a class V antiarrhythmic agent. When adenosine is used to cardiovert an abnormal rhythm, it is normal

for the heart to enter ventricular asystole for a few seconds. This can be disconcerting to a normally conscious patient, and is associated with angina-like sensations in the chest.[9]

Nuclear stress test

Adenosine is used as an adjunct to thallium (TI 201) or technetium (Tc99m) myocardial perfusion scintigraphy (nuclear stress test) in patients unable to undergo adequate stress testing with exercise.[10]

Dosage

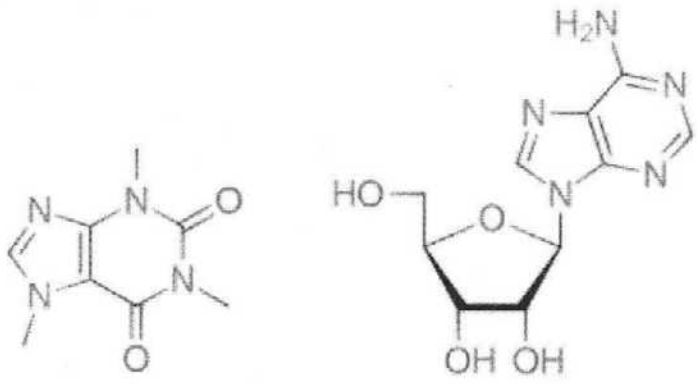
When given for the evaluation or treatment of a supraventricular tachycardia (SVT), the initial dose is 6 mg to 12 mg, depending on standing orders or provider preference,[11] given as a rapid parenteral infusion. Due to adenosine's extremely short half-life, the IV line is started as proximal (near) to the heart as possible, such as the antecubital fossa. The IV push is often followed with an immediate flush of 10-20 ccs of saline. If this has no effect (i.e., no evidence of transient AV block), a dose of 12 mg can be given 1–2 minutes after the first dose. When given to dilate the arteries, such as in a "stress test", the dosage is typically 0.14 mg/kg/min, administered for 4 or 6 minutes, depending on the protocol.

The recommended dose may be increased in patients on theophylline, since methylxanthines prevent binding of adenosine at receptor sites. The dose is often decreased in patients on dipyridamole (Persantine) and diazepam (Valium) because adenosine potentiates the effects of these drugs. The recommended dose is also reduced by half in patients presenting congestive heart failure, myocardial infarction, shock, hypoxia, and/or hepatic or renal insufficiency, and in elderly patients.

Drug interactions

Dipyridamole potentiates the action of adenosine, requiring the use of lower doses.

Methylxanthines (e.g., caffeine, found in coffee, or theophylline in tea, or theobromine, as found in chocolate) competitively antagonize adenosine's effects; an increased dose of adenosine may be required. By nature of caffeine's purine structure,[12] it binds to some of the same receptors as adenosine.[12] The pharmacological effects of adenosine may be blunted in individuals taking large quantities of methylxanthines.[13]



Caffeine's principal mode of action is as an antagonist of adenosine receptors in the brain.

Contraindications

Common contraindications for adenosine include

- Asthma, traditionally considered an absolute contraindication. This is being contended and it is now considered a relative contraindication (however, selective adenosine antagonists are being investigated for use in treatment of asthma)[14]
- Decompensated heart failure
- Long QT syndrome
- Poison/drug-induced tachycardia
- Second- or third-degree heart block (without a pacemaker)
- Severe hypotension
- Sick sinus syndrome (without a pacemaker)

When administered via a central lumen catheter, adenosine has been shown to initiate atrial fibrillation because of its effect on atrial tissue. In individuals with accessory pathways, the onset of atrial fibrillation can lead to a life-threatening ventricular fibrillation. However, adenosine may be administered if equipment for cardioversion is immediately available as a backup.

Side effects

Many individuals experience facial flushing, a temporary rash on the chest, lightheadedness, diaphoresis, or nausea after administration of adenosine due to its vasodilatory effects. Metallic taste is a hallmark side-effect of adenosine administration. These symptoms are transitory, usually lasting less than one minute. It is classically associated with a sense of "impending doom", more prosaically described as apprehension. This lasts a few seconds after administration of a bolus dose, during transient asystole induced by intravenous administration. In some cases, adenosine can make patients' limbs feel numb for about 2–5 minutes after administration intravenously depending on the dosage (usually above 12 mg).

Pharmacological effects

Adenosine is an endogenous purine nucleoside that modulates many physiological processes. Cellular signaling by adenosine occurs through four known adenosine receptor subtypes (A_1, A_{2A}, A_{2B}, and A_3).[15]

Extracellular adenosine concentrations from normal cells are approximately 300 nM; however, in response to cellular damage (e.g. in inflammatory or ischemic tissue), these concentrations are quickly elevated (600–1,200 nM). Thus, in regard to stress or injury, the function of adenosine is primarily that of cytoprotection preventing tissue damage during instances of hypoxia, ischemia, and seizure activity. Activation of A_{2A} receptors produces a constellation of responses that in general can be classified as anti-inflammatory.[16]

Adenosine receptors

All adenosine receptor subtypes (A_1, A_{2A}, A_{2B}, and A_3) are G-protein-coupled receptors. The four receptor subtypes are further classified based on their ability to either stimulate or inhibit adenylate cyclase activity. The A_1 receptors couple to $G_{i/o}$ and decreases cAMP levels, while the A_2 adenosine receptors couple to G_s, which stimulates adenylate cyclase activity. In addition, A_1 receptors couple to G_o, which has been reported to mediate adenosine inhibition of Ca^{2+} conductance, whereas A_{2B} and A_3 receptors also couple to G_q and stimulate phospholipase activity. Researchers at Cornell University have recently shown adenosine receptors to be key in opening the blood-brain barrier (BBB). Mice dosed with adenosine have shown increased transport across the

BBB of amyloid plaque antibodies and prodrugs associated with Parkinson's disease, Alzheimer's, multiple sclerosis, and cancers of the central nervous system.[17]

Ghrelin/growth hormone secretagogue receptor

Adenosine is an endogenous agonist of the ghrelin/growth hormone secretagogue receptor.[18] However, while it is able to increase appetite, unlike other agonists of this receptor, adenosine is unable to induce the secretion of growth hormone and increase its plasma levels.[18]

Mechanism of action

When it is administered intravenously, adenosine causes transient heart block in the atrioventricular (AV) node. This is mediated via the A_1 receptor, inhibiting adenylyl cyclase, reducing cAMP and so causing cell hyperpolarization by increasing K^+ efflux via inward rectifier K^+ channels, subsequently inhibiting Ca^{2+} current.[19] It also causes endothelial-dependent relaxation of smooth muscle as is found inside the artery walls. This causes dilation of the "normal" segments of arteries, i.e. where the endothelium is not separated from the tunica media by atherosclerotic plaque. This feature allows physicians to use adenosine to test for blockages in the coronary arteries, by exaggerating the difference between the normal and abnormal segments.

The administration of adenosine also reduces blood flow to coronary arteries past the occlusion. Other coronary arteries dilate when adenosine is administered while the segment past the occlusion is already maximally dilated, which is a process called coronary steal. This leads to less blood reaching the ischemic tissue, which in turn produces the characteristic chest pain.

Metabolism

Adenosine used as a second messenger can be the result of *de novo* purine biosynthesis via adenosine monophosphate (AMP), though it is possible other pathways exist.[20]

When adenosine enters the circulation, it is broken down by adenosine deaminase, which is present in red blood cells and the vessel wall.

Dipyridamole, an inhibitor of adenosine nucleoside transporter, allows adenosine to accumulate in the blood stream. This causes an increase in coronary vasodilatation.

Adenosine deaminase deficiency is a known cause of immunodeficiency.

Research

Viruses

The adenosine analog NITD008 has been reported to directly inhibit the recombinant RNA-dependent RNA polymerase of the dengue virus by terminating its RNA chain synthesis. This suppresses peak viremia and rise in cytokines and prevented lethality in infected animals, raising the possibility of a new treatment for this flavivirus.[21] The 7-deaza-adenosine analog has been shown to inhibit the replication of the hepatitis C virus.[22]

BCX4430 is protective against Ebola and Marburg viruses.[23] Such adenosine analogs are potentially clinically useful since they can be taken orally.

Anti-inflammatory properties

Adenosine is believed to be an anti-inflammatory agent at the A_{2A} receptor.[24][25] Topical treatment of adenosine to foot wounds in diabetes mellitus has been shown in lab animals to drastically increase tissue repair and reconstruction. Topical administration of adenosine for use in wound-healing deficiencies and diabetes mellitus in humans is currently under clinical investigation.

Methotrexate's anti-inflammatory effect may be due to its stimulation of adenosine release.[26]

Central nervous system

In general, adenosine has an inhibitory effect in the central nervous system (CNS). Caffeine's stimulatory effects are credited primarily (although not entirely) to its capacity to block adenosine receptors, thereby reducing the inhibitory tonus of adenosine in the CNS. This reduction in adenosine activity leads to increased activity of the neurotransmitters dopamine and glutamate.[27] Experimental evidence suggests that adenosine and adenosine agonists can activate Trk receptor phosphorylation through a mechanism that requires the adenosine A_{2A} receptor.[28]

Hair

Adenosine has been shown to promote thickening of hair on people with thinning hair.[29][30] A 2013 study compared topical adenosine with minoxidil in male androgenetic alopecia, finding it was not superior to minoxidil and further trials were needed.[31]

Sleep

The principal component of marijuana, delta-9-tetrahydrocannabinol (THC) and the endocannabinoid anandamide (AEA) induce sleep in rats by increasing adenosine levels in the basal forebrain. They also significantly increase slow-wave sleep during the third hour, mediated by CB1 receptor activation. These findings identify a potential therapeutic use of cannabinoids to induce sleep in conditions where sleep may be severely attenuated.[32]

See also

- Adenosine receptor
- Adenosine reuptake inhibitor
- List of growth hormone secretagogues

References

1. "Adenosine" (https://www.drugs.com/monograph/adenosine.html). The American Society of Health-System

PubMed

Format: Abstract

Full text links

Full Text FREE Mol Cancer Ther

PMC FREE Full text

Mol Cancer Ther. 2010 Jan;9(1):236-45. doi: 10.1158/1535-7163.MCT-09-0767. Epub

8-Amino-adenosine inhibits multiple mechanis

This would be serious business. ATP blockage also.
Aminoadenosine is a transcription inhibitor.

PubMed

Format: Abstract

Full text links

Mol Cancer Ther. 2010 Jan;9(1):236-45. doi: 10.1158/1535-7163.MCT-09-0767. Epub

Full Text FREE Mol Cancer Ther PMC FREE Full text

8-Amino-adenosine inhibits multiple mechanisms of transcription.

Frey JA[1], Gandhi V.

Author information

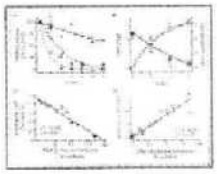
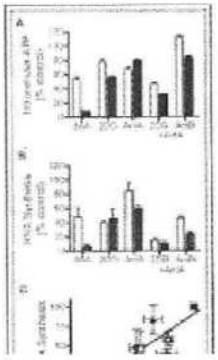
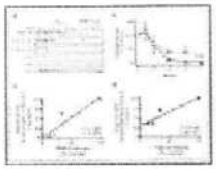
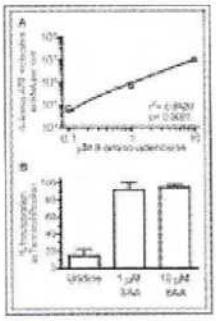
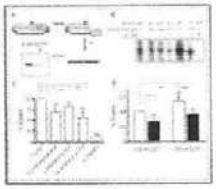
Abstract

Roscovitine and flavopiridol suppress cyclin-dependent kinase 7 (CDK7) and CDK9 activity resulting in transcription inhibition, thus providing an alternative mechanism to traditional genotoxic chemotherapy. These agents have been effective in slow or nonreplicative cell types. 8-Amino-adenosine is a transcription inhibitor that has proved very effective in multiple myeloma cell lines and primary indolent leukemia cells. The objective of the current work was to define mechanisms of action that lead to transcription inhibition by 8-amino-adenosine. 8-Amino-adenosine is metabolized into the active triphosphate (8-amino-ATP) in cells. This accumulation resulted in a simultaneous decrease of intracellular ATP and RNA synthesis. When the effects of established ATP synthesis inhibitors and transcription inhibitors on intracellular ATP concentrations and RNA synthesis were studied, there was a strong correlation between ATP decline and RNA synthesis. This correlation substantiated the hypothesis that the loss of ATP in 8-amino-adenosine-treated cells contributes to the decrease in transcription due to the lack of substrate needed for mRNA body and polyadenylation tail synthesis. RNA polymerase II COOH terminal domain phosphorylation declined sharply in 8-amino-adenosine-treated cells, which may have been due to the lack of an ATP phosphate donor or competitive inhibition with 8-amino-ATP at CDK7 and CDK9. Furthermore, 8-amino-ATP was incorporated into nascent RNA in a dose-dependent manner at the 3'-end resulting in transcription termination. Finally, in vitro transcription assays showed that 8-amino-ATP competes with ATP for incorporation into mRNA. Collectively, we have concluded that 8-amino-adenosine elicits effects on multiple mechanisms of transcription, providing a new class of transcription inhibitors.

PMID: 20053765 PMCID: PMC2806509 DOI: 10.1158/1535-7163.MCT-09-0767

[Indexed for MEDLINE] Free PMC Article

Images from this publication. See all images (6) Free text


Building Blocks of Life

Self Replicating Biological Molecule

Amino adenosine - Ester

Chemists Make Molecule With Hint of Life - The New York Times https://www.nytimes.com/1990/10/30/science/chemists-make-mole...

The New York Times https://nyti.ms/29wBKBa

ARCHIVES | 1990

Chemists Make Molecule With Hint of Life

By MALCOLM W. BROWNE OCT. 30, 1990

MOLECULES that can process raw materials to make copies of themselves are essential components of all living things but are very rare elsewhere. For the first time, however, scientists have begun to synthesize self-replicating molecules in the laboratory.

Although these synthetic molecules are far simpler than most of the natural biological ones, they mimic important living processes and may offer clues to how organic chemicals acquired the ability to duplicate themselves billions of years ago, setting the stage for life.

Some scientists believe that an understanding of how such molecules work may eventually shed light on how certain diseases are transmitted. A related class of synthetic molecules may also lead to new techniques for manufacturing drugs and substitutes for the oxygen-carrying component of blood.

One of the scientists studying self-replicating molecules is Dr. Julius Rebek Jr., a chemist at the Massachusetts Institute of Technology. Earlier this year a team headed by Dr. Rebek announced the creation of the compound called amino adenosine triacid ester (AATE), which had an astonishingly lifelike ability

simpler molecules, holds them in positions in which they can join together, and then releases the newly assembled molecule, which is a duplicate of itself, Dr. Rebek explained.

The key to this process is a hydrogen bond, a type of molecular link in which a hydrogen atom shares its single electron with two other atoms, either in the same molecule or in two different molecules, creating a link between them.

A hydrogen bond forms, breaks and reforms millions of times each second, and is much less stable than another typical molecular bond, the covalent bonds found in most inorganic compounds, in which each atomic partner contributes an electron to produce a strong bond consisting of an electron pair.

"We spent three years measuring the properties and effects of these hydrogen bonds before we were able to create template molecules that replicate themselves," Dr. Rebek said. The relatively weak hydrogen bonds on which the replication process is based work like zippers that can temporarily hold molecules in place and then release them.

The two ingredients used in the experiment were an ester (a substance made from an alcohol and an organic acid) and amino adenosine. Once a few molecules of AATE have been made from these ingredients, the AATE molecules can serve as templates for making replicas of themselves.

After each template has assembled two smaller molecules into a new template, the latter breaks its hydrogen bonds with its creator, peels away and begins its own life as a template. The replication process rapidly accelerates from then on, until all the raw materials are used up.

<> <> Although AATE does not itself participate in living processes, its structure and chemical behavior are analogous to many compounds that do. Among them are DNA and RNA, molecules that encode information essential to the reproduction of living cells and multicellular organisms. In these genetic molecules, as in AATE, easily broken hydrogen bonds are a vital feature of the self-replication process.

The synthesis of the new compound does not suggest that life originated with this particular molecule. But chemists believe that the molecules from

Another vital function of certain biological molecules is their ability to catalyze reactions. Catalysts accelerate chemical reactions without being consumed themselves, and it appears that scientists are now close to understanding how the molecules that were precursors of life could have developed both the ability to replicate themselves and to catalyze replication and other processes.

In the study of this prebiotic chemistry, Dr. Rebek said, a central issue "has been the question of what kind of molecule started things off."

He said scientists "have pretty much settled on nucleic acids, particularly RNA, because it's easy to visualize how RNA can act as a template for its own formation, just as it does in living systems even today." RNA, or ribonucleic acid, carries the chemical instructions needed by living cells to synthesize the proteins on which all organisms are based.

But it took a second discovery to establish RNA as a probable precursor of life: a discovery for which the Nobel Prize in Chemistry was awarded last year to Dr. Thomas R. Cech of the University of Colorado and Dr. Sidney Altman of Yale University. In independent studies, they found that RNA not only provides the information needed to make proteins, but it also catalyzes protein production.

"Prior to that discovery," Dr. Rebek said, "everyone thought that only proteins and enzymes made of proteins could act as catalysts in living systems."

Molecules 'Teach Us Something'

Dr. Cech himself has reservations about asserting that RNA is unique in having triggered the chemistry from which which life sprang.

"One cannot answer a question about the history of life by doing reconstruction experiments in the laboratory today," he said in an interview. "One can only say what is chemically reasonable, what is plausible, what are some of the principles that were likely to have been involved in the origin of life."

With that reservation, he said, study of synthetic analogues of biological molecules of the kind created by Dr. Rebek "teach us something about real fundamental principles of self-organization of molecules."

Although Dr. Cech believes that it is impossible to determine which

"There are formidable problems yet to be solved," Dr. Cech said, "but I think it's quite likely that these individual steps will be performed in simple systems in the laboratory within the next 5 or 10 years."

Up to now, no proteins have been found that are capable of serving as templates in their own replication. But Dr. Rebek believes that it may be possible to create such proteins in the laboratory. He and Dr. Alex Rich of M.I.T.'s biology department are working on the synthesis of several potential candidates. Success could imply the possibility that certain living processes might arise from proteins alone, without the usual directions provided by RNA. Self-Assembling Molecules

In another branch of chemical research, scientists are experimenting with a class of molecules that assemble themselves automatically from simple components without the use of templates.

Dr. David S. Lawrence and his colleagues at the State University of New York at Buffalo are creating self-assembling molecules by selecting component molecules whose parts carry different electric charges or are either attracted to or repelled by water. If mixtures of such molecules are organized correctly, the molecules snap together automatically, like a self-assembling jigsaw puzzle.

Many such molecules are known in nature. Among them is the hemoglobin of red blood cells, which can assemble itself spontaneously from its four molecular components. But only rarely have scientists devised such self-assembling processes in the laboratory.

Among the chemicals Dr. Lawrence has created by such reactions are cyclodextrin compounds -- cage-like molecules that can hold metal atoms in their centers.

One such molecule contains an iron atom and is structurally quite similar to heme, the working part of the hemoglobin molecule that captures oxygen for transport from the lungs to the body's tissue. Dr. Lawrence and Dr. Rich are investigating some new self-assembling compounds that contain cobalt instead of iron, and which might one day be used in artificial blood.

For more than a decade, scientists in Japan and the United States have experimented with an artificial blood based on a class of compounds called

bind oxygen drawn from the lungs and transport it to the tissues that need it.

But although the blood substitute has been successfully used in many patients, it has undesirable side effects and does not perform as well as real blood as an oxygen carrier. Many chemists believe that a truly successful blood substitute must await the development of some oxygen carrier that closely resembles real heme. Synthetic Hemoglobin

Dr. Lawrence's research is supported by the American Heart Association, but he said the Navy is also interested in the possibility of developing synthetic hemoglobin. Natural blood has a limited storage life, and on long voyages, including those of space ships, a synthetic oxygen-carrier equivalent to natural hemoglobin could save lives, he said.

"You can't inject hemoglobin directly into the blood, because it comes apart and poisons the kidneys," Dr. Lawrence said. "But it may be possible to encapsulate some synthetic oxygen-carrier similar to the heme of hemoglobin in liposomes." Synthetic liposomes are tiny hollow spheres of protein sometimes used in scratch-and-sniff advertisements. Scratching paper coated with a myriad of liposomes ruptures many of the little spheres and releases the substance they contain.

"Liposomes containing synthetic hemoglobin might be injected into the blood stream," Dr. Lawrence said, "and oxygen from the lungs would suffuse into each sphere, where the compound would bind it and carry it to the tissues. In that way, the toxic hemoglobin substitute inside the liposomes would not come into contact with the blood itself, even though it was carried through the blood stream."

One mission of today's chemists, Dr. Lawrence said, is to devise new molecules that can recognize each other and assemble themselves into complex entities without external instructions.

"In a sense, a human being is a self-assembly of many complex molecules from simple building blocks," he said. "That is the kind of ideal at which we're aiming." Copyists's Skills at the Molecular Level A chemical called AATE is a molecular template that holds two simpler molecules in positions that let them

simpler molecules in place as they are assembled. The new molecule breaks away from its template and begins acting as a template on its own. (source: Julius Rebek Jr.) (pg. C7)

A version of this article appears in print on October 30, 1990, on Page C00001 of the National edition with the headline: Chemists Make Molecule With Hint of Life.

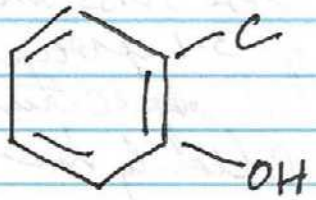
I am now looking @ the precipitate that settled in Tube 3-1.

I have centrifuged the material and have dissolved it w/an 1M HCl. It is a very small sample, ~ 1 mL.

I have run a UV plot. There are absorbance peaks @ ~ 270 nm and ~ 230 nm.

Now let's go for NIR:

We have absorbance @ 960 nm.
Since we have used H_2O as a blank, this suggests ArOH
This is very consistent w/ our analysis.
It also does look like we have absorbance taking place near 1100. This is ArCH



ArOH & ArCH are consistent w/ IR database search.

Just recall this is the precipitate, not the solution.

While we are @ it, let's gather NIR for the SOLUTION of Tube 3-1!

We have absorbance @ 1080. This is ArCH.
Also a very strong slope break (but not a peak) @ ~ 960.
This also is ArOH.

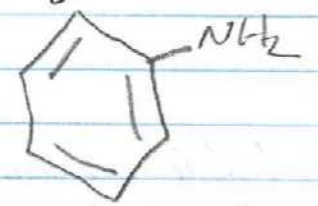
These results actually are similar strength, w/ the precipitate.

Now let's conduct protein colorimetric test of the precipitate dissolved in 1M HCl for protein.

Results of the test are negative.

Now for IR:

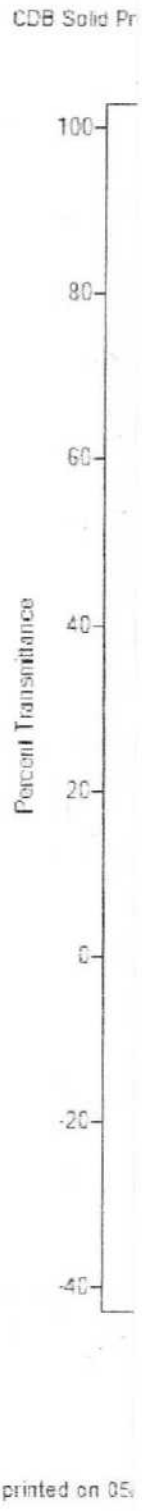
We learn that Tube 3-1 precipitate is essentially identical to that of 3-1 solution. No need to further distinguish the two. The precipate (dissolved in moderate strength HCl) did leave a yellow film on the ATR plate so a thin film nature is important to record here.

Peaks for the Precipitate	vs Solution	From SDBS analysis of 3-1 precipitate IR spectrum, we clearly have:
3393		
3353	3354	
3242	3242	
1630	1634	
1288 weak	1275 weak	
1086 weak	1142 weak	
691	646	

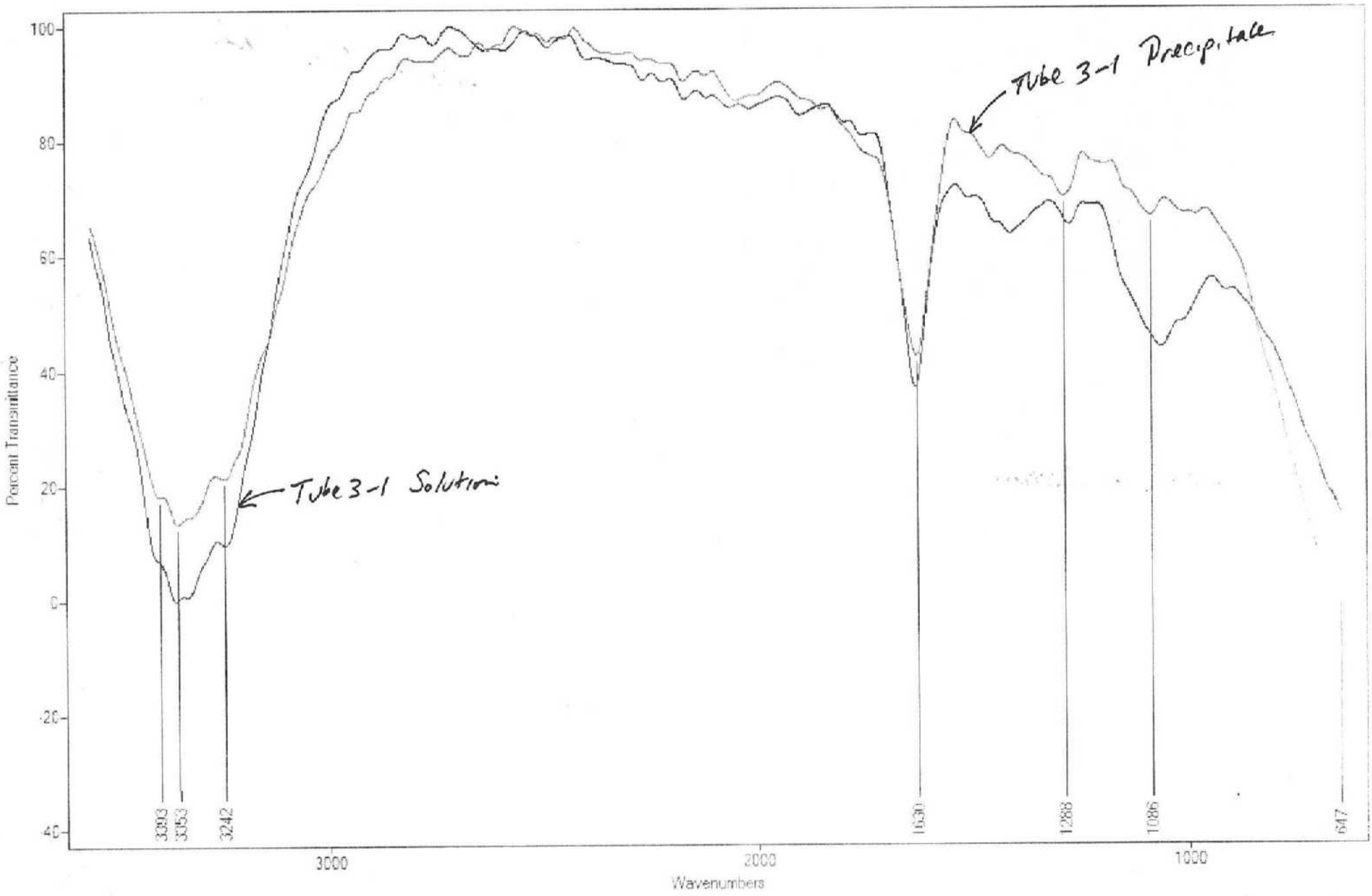
present

NIR also gave OH, and Ar-C

Tube 3-1 Precipitate + 3-1 Solution Compared.



CDB Solid Protein - LC - Tube 3-1 - PRECIPITATE Dissolved in HCl - May 28 2019 - 13.spc: 05/29/2019 00:32:08 Idle



printed on 05/29/2019 00:34:54

May 29 2019

A very important confirmation test for Tube 3-1 has taken place today.

A positive colorimetric test for the presence of an amine using ninhydrin on Tube 3-1 has taken place!

The confirm from all tests that Tube 3-1 Contained, at least a monomeric structure

AN AROMATIC POLAR AMINE COMPOUND.
(recall also that it is highly conductive.)
Our source of information to synthesize are.

1. Protein colorimetric test (negative result)
2. Numerous highly probable functional groups from the SDBS IR database, including the amine group.
3. Near-Infrared Analysis
   We have both ArOH & ArCH
   ~~Compromised~~ indicated.
4. Ninhydrin test - positive for amine.
5. Significant IR analysis is available to assess

It is also of interest to note that the ninhydrin gave a weak, but observably detectable signal. This means that our amine concentration in the LC separation is fairly low, and this is most unexpected.

IR shows itself to be the most sensitive detection method.

The amazing part is that we were able to complete it all by the method. All are dealing w/ small samples here, low concentrations, & bacteria cultures as the base for all of the work.

The greatest unknown now is the questions of LC Trial #2 on Tube 2-1 IR analyses which indicate pot candidates of

1. C-N aliphatic amine
2. sulfonic ester
3. ester

(Recall our elf assembly paper?)

There additional effort needs to be expended here to sort through our various functional group sources of information.

Let's continue, in the interim while the column setup remains, to proceed once again w/ the use of acetic acid as the eluent.

I must watch the column carefully this time.

Released values
pH 8.78
ORP +196

Tube 3-3

t	EC	pH	ORP	Brix	Solvent
1535	Not Available	5.65	+345 (max)	2.0	Vinegar

Approx only 5 ml is captured in the sample
ORP clamp @ maximum ~~clocked~~ in base of collector.

There is an interesting activation w/ Tube 3-3
Notice the lack of hydrocarbons. These
indicate an inorganic nature, it might
seem.
Acid is certainly a lost descriptive
Peaks are @: to the column.

med 1553 cm^{-1} Pyrimidines (1520-1580) C=N

str 1414 Sulfonic ester (RO_2SO_2) S=O

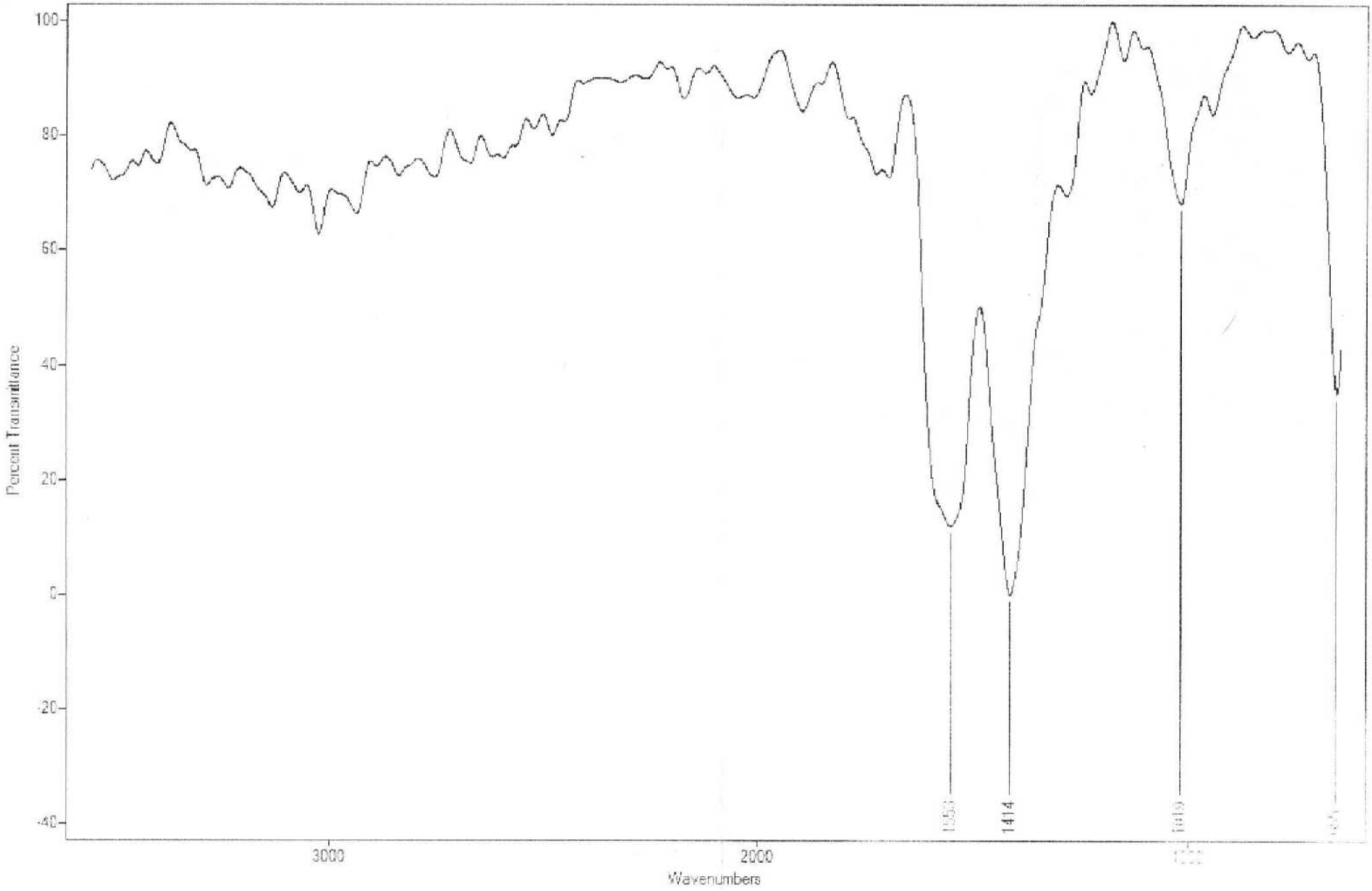
1019 C-F monofluoro derivates (1000-1110)
methylene acetal (C-O) ~1040
655 Bromo derivatives C-Br ~655

There is indeed a curious affair. What are
Pyrimidenes?
Let's look w/ NIR:

Our closest match to the spectrum is Tube 1-5.
It looks to be an identical match except without
the amine structure.

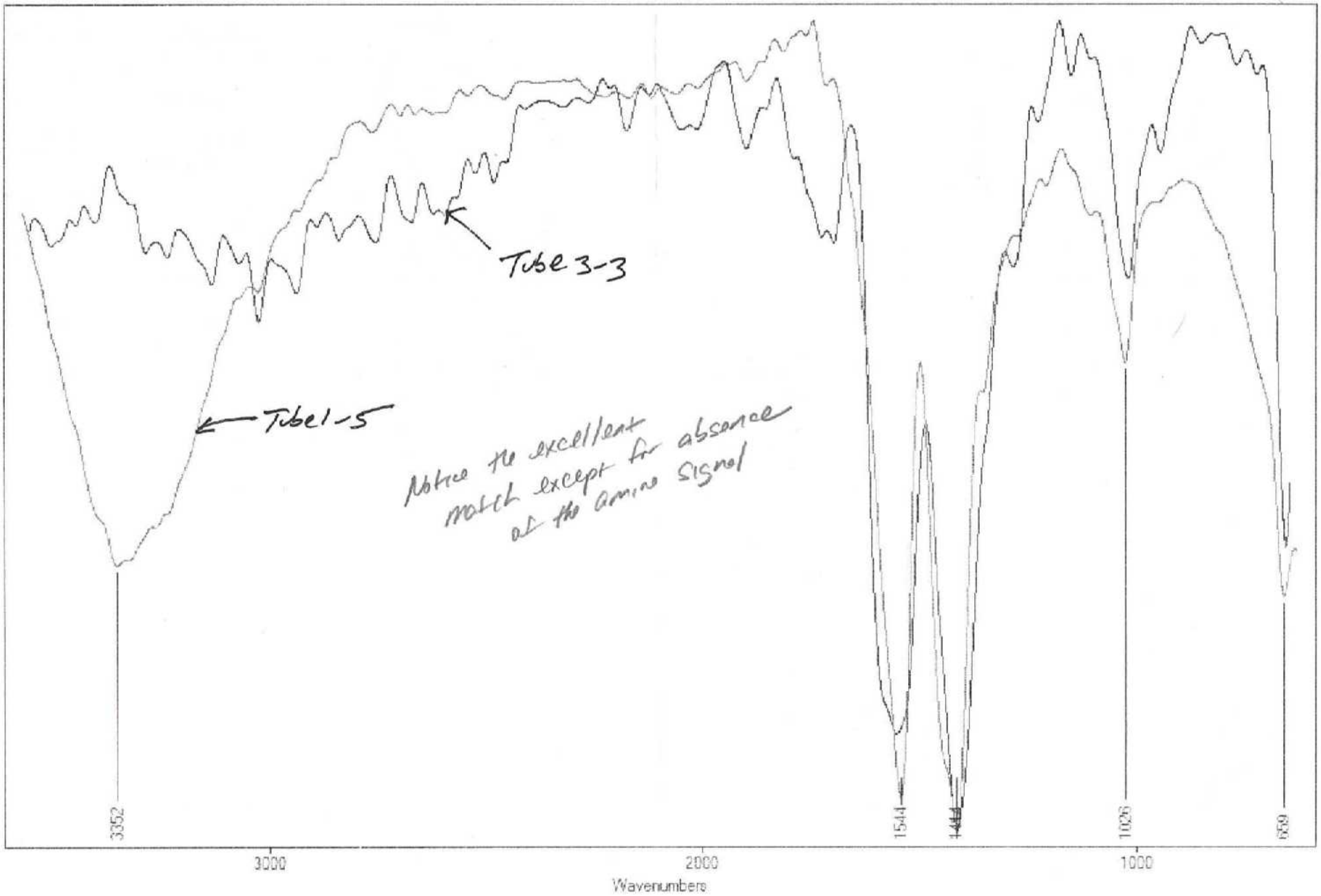
Compare these two plots - w/ & w/Nt an amine??

CDB Solid Protein - LC - Tube 3-3 - 5 ml Vinegar - May 28 2019 - 14.spc: 05/29/2019 17:38:52 title



printed on 05/29/2019 17:40:57

CDB Solid Protein - LC - Tube 1-5 - May 26 2019 - 08.spc: 05/26/2019 15:34:38 title



printed on 05/29/2019 18:05:51

Discard

Tube 3-4	t	EC	pH	ORP	Brix	Solvent
	1650	4.70	4.69	315	3.0	Vinegar

IR is same as 3-3 — Discard
NIR gives us a very slight, and I do mean slight absorbance peak @ ~960nm

This suggests either water or ArOH.
We use H_2O as a blank but since the signal is so slight it may truly only represent some type of differential detecting of H_2O.

The case of ArOH cannot be justified at this time due to the lack of detection of hydrocarbons. The shape of the weight of NIR detection toward the differential detection of water, at the least the NIR spectrum provides no showing data

The LC column does can separate itself from acidic disturbances when dis is flushed out of the columns.

We may only be dealing w/ the C=N bond and the S=O bond — it may well not be appropriate to extend the consideration to pyrimidines since no hydrocarbons are observed. To Notice the relationship to DNA, however.

Purines vs. Pyrimidines

Purines and **Pyrimidines** are nitrogenous bases that make up the two different kinds of nucleotide bases in DNA and RNA. The two-carbon nitrogen ring bases (adenine and guanine) are purines, while the one-carbon nitrogen ring bases (thymine and cytosine) are pyrimidines.

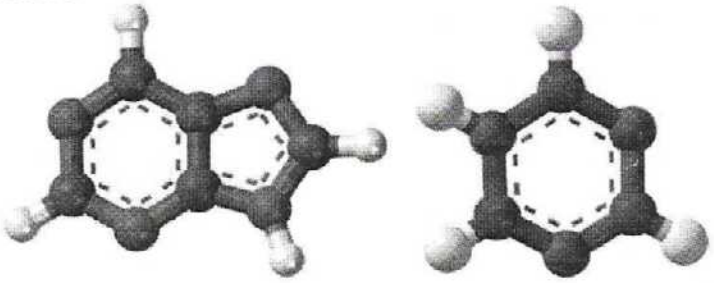
Comparison chart

Differences — Similarities —

	Purines	Pyrimidines
Introduction (from Wikipedia)	A purine is a heterocyclic aromatic organic compound, consisting of a pyrimidine ring fused to an imidazole ring.	Pyrimidine is a heterocyclic aromatic organic compound similar to benzene and pyridine, containing two nitrogen atoms at positions 1 and 3 of the six-member ring. It is isomeric with two other forms of diazine.
Function	Production of RNA and DNA, proteins and starches, the regulation of enzymes and cell signaling.	Production of RNA and DNA, proteins and starches, the regulation of enzymes and cell signaling.
Nucleobases	Adenine and guanine	Cytosine, thymine, uracil
Structure	A pyrimidine ring fused to a imidazole ring. Contains two carbon-nitrogen rings and four nitrogen atoms.	Contains one carbon-nitrogen ring and two nitrogen atoms.
Melting point	214 °C, 487 K, 417 °F	20–22 °C
Type of Compound	Heterocyclic aromatic organic compound	Heterocyclic aromatic organic compound
Molecular formula	C5H4N4	C4H4N2
Molar mass	120.11 g mol−1	80.088 g mol-1
MeSH	Purine	Pyrimidine
SMILES	c1c2c(nc[nH]2)ncn1	C1=CN=CN=C1
CAS number	120-73-0	289-95-2 Y
PubChem	1044	9260
Synthesis in Lab	Traube Purine Synthesis	Biginelli Reaction

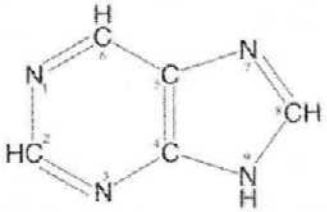
Contents: Purines vs Pyrimidines

Structure



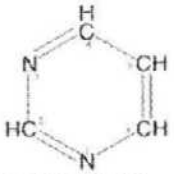
Purine (L) and Pyrimidine (R) molecules, where Black= Carbon, White=Hydrogen, Blue=Nitrogen

A purine is a heterocyclic aromatic organic compound containing 4 nitrogen atoms. It contains two carbon rings, and is made of a pyrimidine ring fused to an imidazole ring.



Structure of a purine

A pyrimidine is a heterocyclic aromatic organic compound containing 2 nitrogen atoms. It contains only one carbon ring.



Structure of a pyrimidine

Function

Both purines and pyrimidines have the same function: they serve as a form of energy for cells, and are essential for production of DNA and RNA, proteins, starch, regulations of enzymes, cell signaling.

	t	EC	pH	ORP	Brix	Solvent
Tube 3-5	1730	5.59	4.75	287	2.3	Vinegar
Tube 3-6	1045	10.2	5.2	261	NA	Vinegar

The Column has once again separated itself. It appears the acid breaks down the medium sand & becomes a fine sand texture.

Tube 3-5 = Tube 3-4 = Tube 3-3

~~Tube~~ Tubes 3-4 & 3-5 are therefore removed.

Excellent Separation Here ↑

Tube 3-6 is significantly different. I noticed that Conductivity increased markedly to a quick maximum and I captured that separation.

Notice this occurred even after vinegar had been used for some time.

This shows that a single solvent can sometime produce several distinct separations given enough time and with careful attention to any change w/in the detectors. Only a single detector picked up the change.

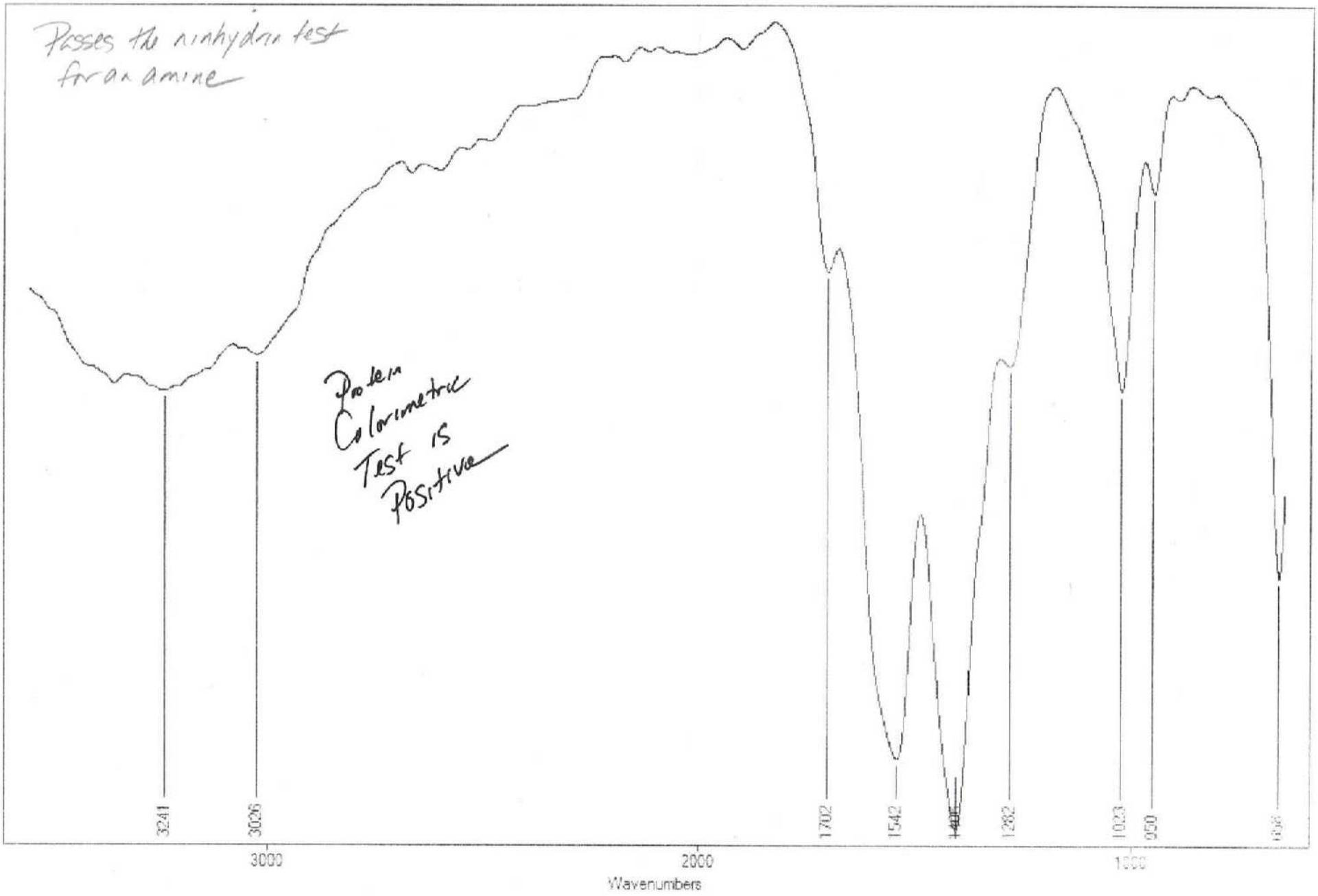
Strangely enough, it looks like vinegar actually cond. from the columns to perform better.

Tube 3-6 is an excellent separation. It seems to best represent the series of Tubes 3-3 to 3-6 (four collections)

CDB Solid Protei

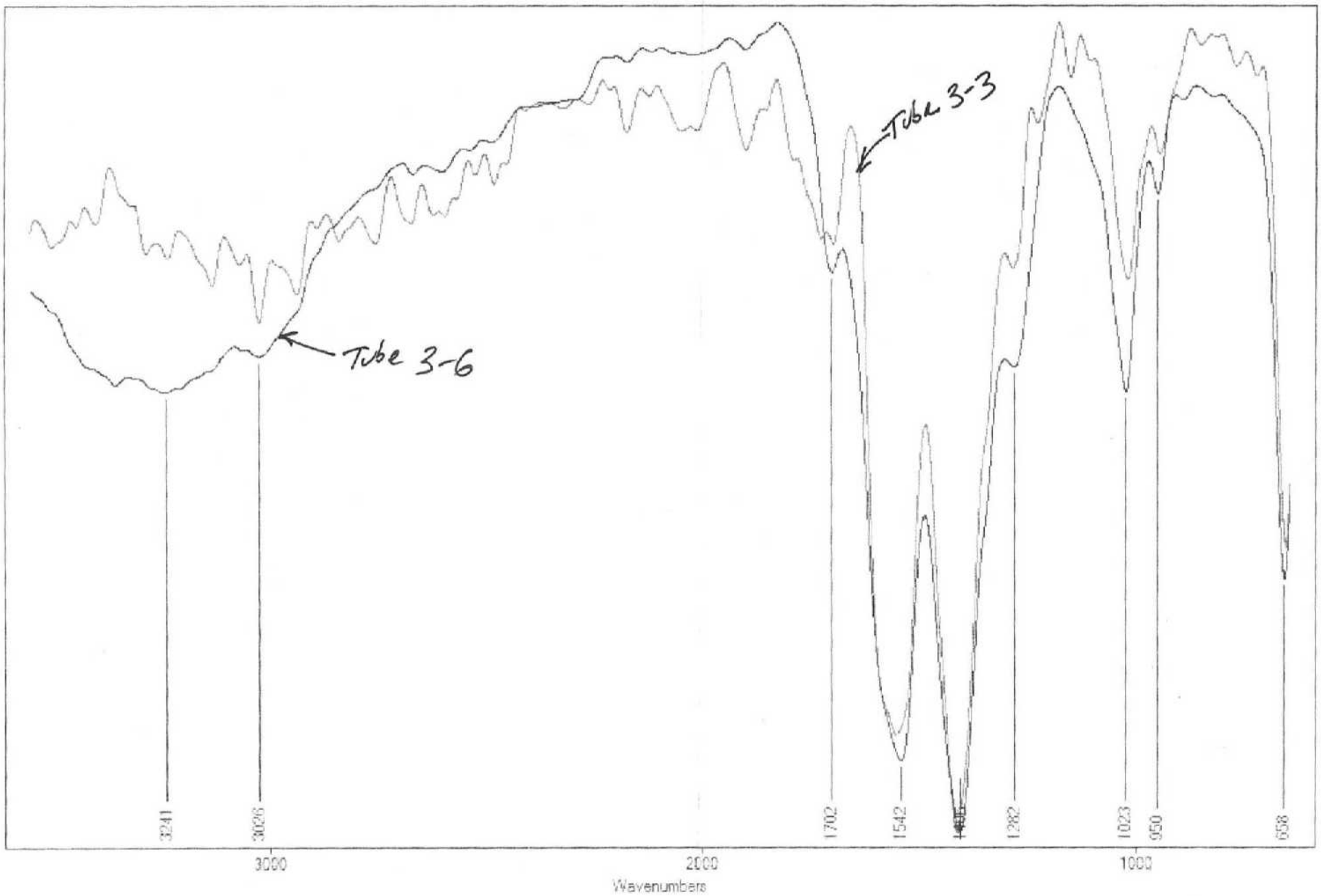
printed on 05/

CDB Solid Protein - LC - Tube 3-6 - Vinegar Solvent - High Conductivity - May 28 2019 - 15 spc: 05/30/2019 01:12:44 title



printed on 05/30/2019 01:23:39

CDB Solid Protein - LC - Tube 3-6 - Vinegar Solvent - High Conductivity - May 28 2019 - 15.spc: 05/30/2019 01:12:44 title



eFTIR

I think that a rather effective "detector" is the flow rate. Most any compound or effluate change produces a change in the flow rate. There is an easy quantity to measure w/ not any equipment but a watch in place.

The size of the drops is another indicator.

I think that a visual indicator can be developed of the form:

Visual Detector = $\frac{\text{Drops per minute}}{\text{Size of Drops}}$

Size of Drops: 1 = small 2 = medium 3 = full + large

Example: $\frac{10 \text{ drops/min}}{1 \text{ (small drop)}} = 10$ $\frac{1 \text{ drop/min}}{3 \text{ (large drop)}} = 0.33$

I think that this would work fairly well in the absence of instrumentation.

Table 3-1	t / EC	pH	ORP	Brix	Flow Detector	Solvent
0125	1325 / 0.01	7.29	+241		11/3 = 3.7	HCl
0135	1335 / 0.43	7.02	165		7/3 = 2.3	
0145	1345 / 4.39	6.77	121		13/3 = 4.3	

You do not really need Brix (index of refraction) Flow detector alone is probably sufficient. Electronic detectors only an asset above that.

Protein

Perform Colorimetric Test Here

Tube3-6 t	EC	pH	ORP	Flow Det.	Solvent
0240	6.86	6.92	84	10/3 = 3.3	HCl

Amine Ninhydrin Test is negative – Not a surprise

Tube 3-7 in water. ~~No IR signal~~. FALSE! There is a strong signal.

Observation in spare column: Vinegar treatment of column followed by ammonia immediately released dye that had been locked up in the column for 3 days.

Correction: Tube 3-7 has a significant IR signal. IR Plot on next page.

Strong signals for amine, also hydrocarbon present. There ~~also~~ are also significant shifts in wavelength from Tube 3-6.

Do not dismiss Tube 3-7. Compare carefully w/ Tube 3-6.

Tube3-9 t	EC	pH	ORP	Flow Det	Solvent
Discard 0240	1.38	7.04	84	5/3 = 1.7	HCl

We lost some of the IR signal here. Tube 3-8 is the strongest

Perform Colorimetric ~~test~~ here

Tubes 3-8 & 3-9 produce a film on the ATR plate. All signs say there is a major protein.

Perform Colorimetric test here.

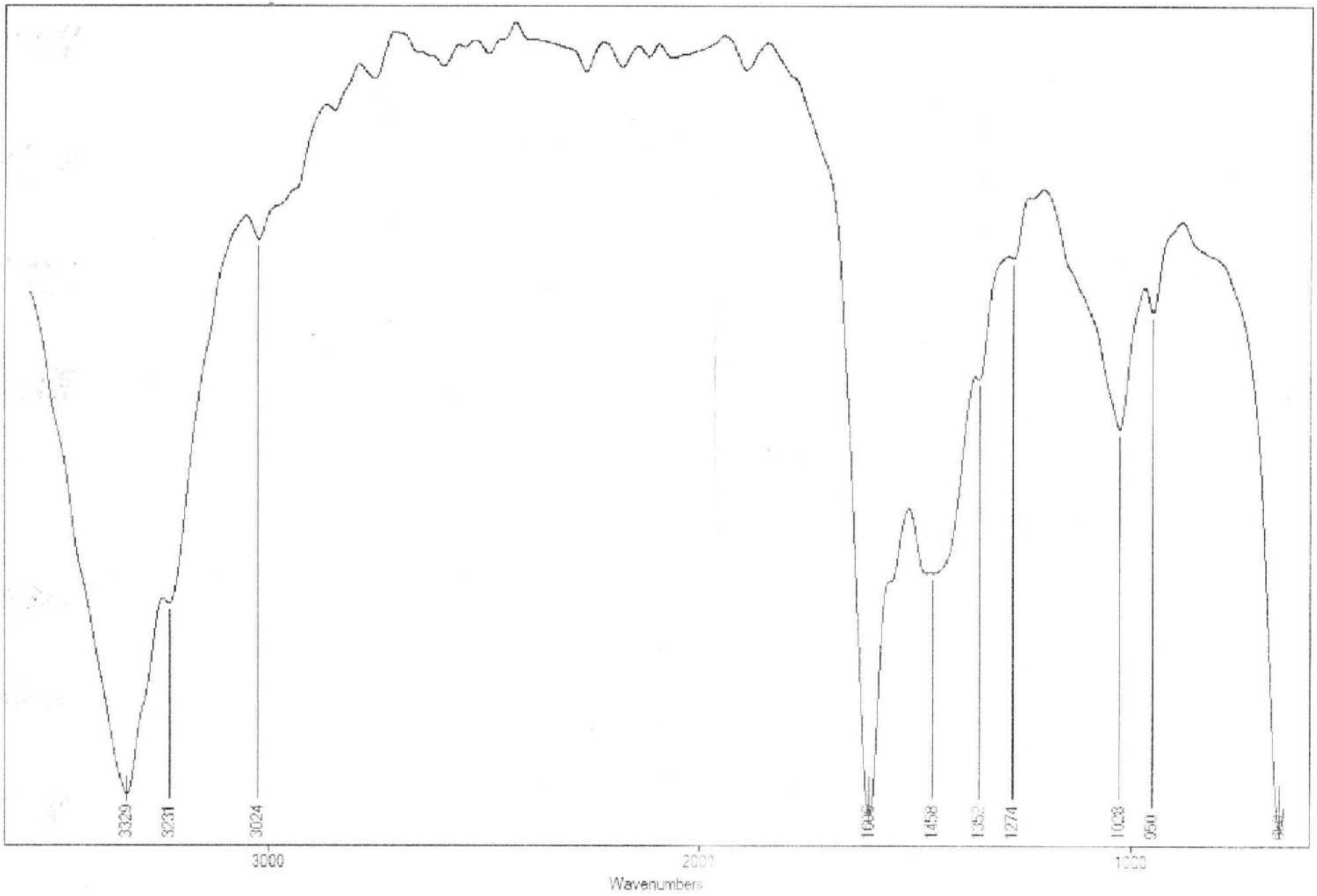
(discarded) Tube 3-1 in HCl. Signal and signal here

Tube 3-8 in HCl " " "

CDB Solid Protein - LC

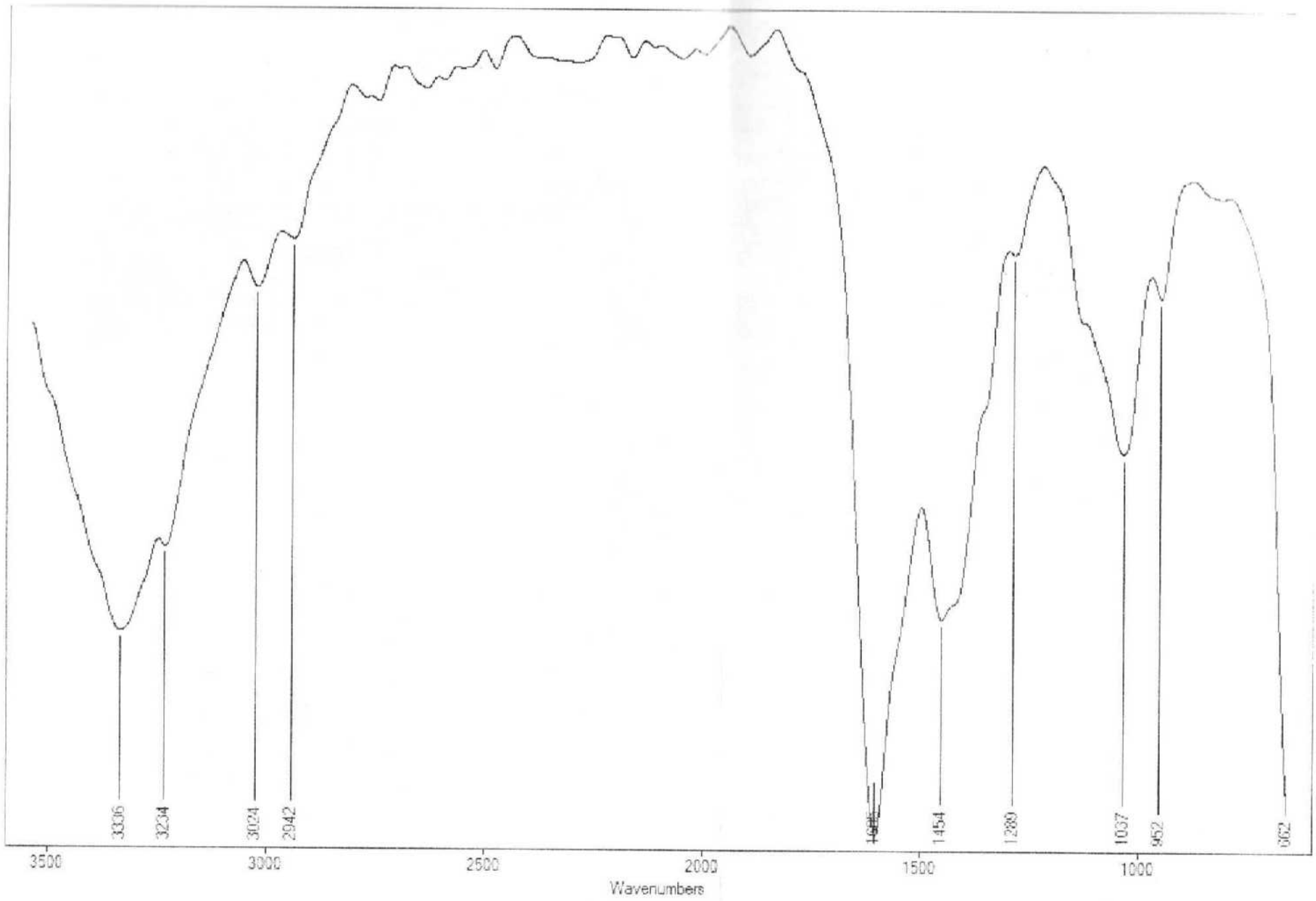
printed on 05/30/2

CDB Sblic Protein - LC - Tube 3-8 - HCl Solvent - May 28 2019 - 17.spc: 05/30/2019 03:48:34 title



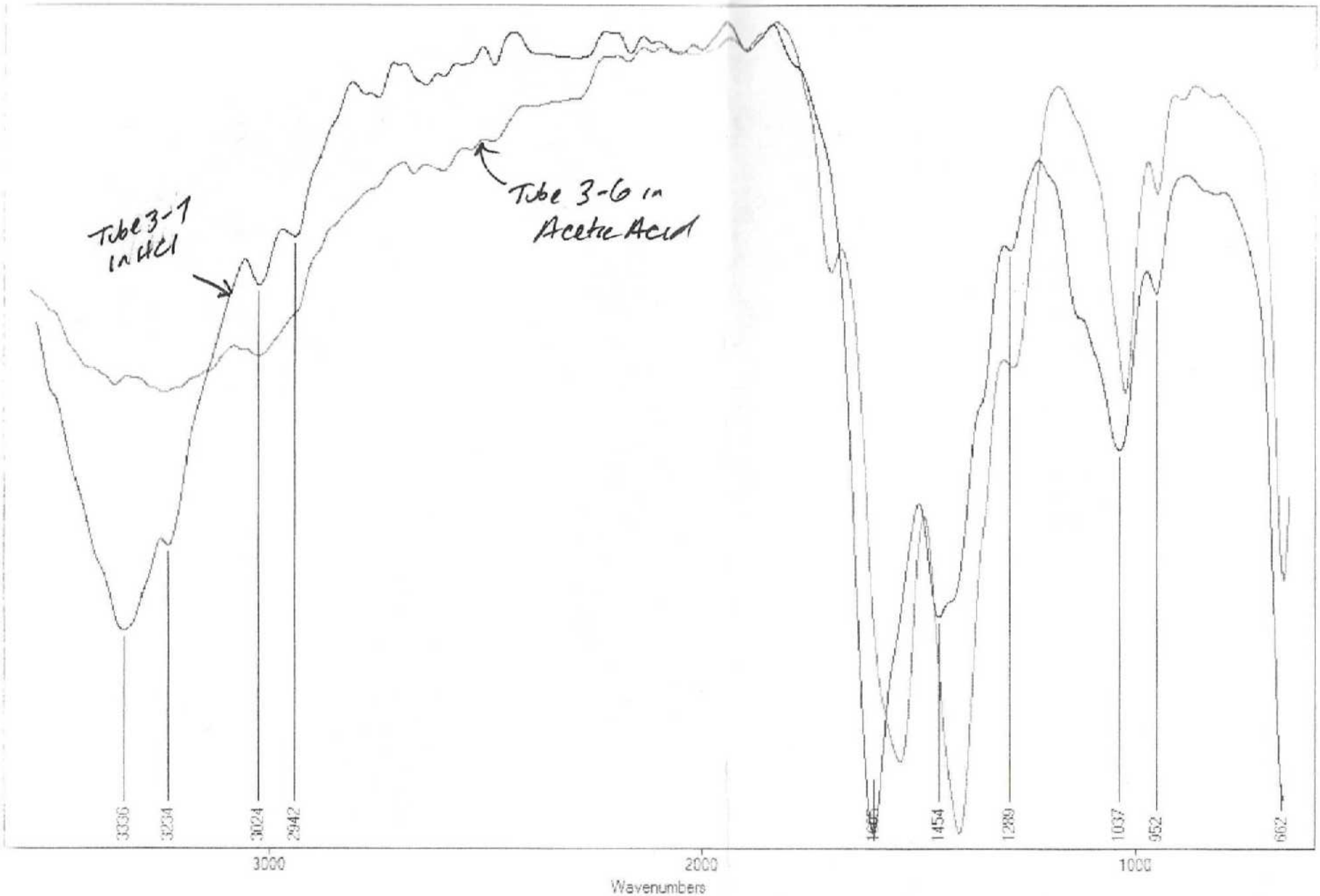
printed on 05/30/2019 03:50:37

Protein - LC - Tube 3-7 - HCl Solvent - May 28 2019 - 16.spc: 05/30/2019 03:31:59 title



05/30/2019 03:34:58

eFTIR


Tube 3-7 in HCl
Tube 3-6 in Acetic Acid
3336
3234
3024
2942
1454
1289
1037
952
662
3000
2000
1000
Wavenumbers

May 31 2019

At this point, Tube 3-8 fails the colorimetric test for protein. This is a bit surprising to me. We obviously have a very good IR spectrum that has been obtained from it.

Recall also that the ammonium test - colorimetric also gives a negative result.

The question of sufficient concentration to detect colorimetrically will also be here.

I send the same negative colorimetric result for protein with Tube 3-9.

Concentration levels? IR spectral interpretations are required.

Let's look @ NIR:

(Incidentally, it seems pretty clear that vinegar flushes are actually a very good method of conditioning the Sand Columns. It eventually dramatically improves the flow rate of the columns) All three columns are now working extremely well.

With NIR, we see that Tube 3-8 & 3-9 appear to be identical, as we expected. We have absorbance @:

1080 (Slight) → Ar CH
960 (strongest) → Ar OH
880 (Slight) → Ar CH

Tubes 3-8 & 3-9 therefore present a very interesting case. The IR interpretation here is going to be crucial.

The determination of amide bond presence and confirmation is going to a critical point of distinction when the IR spectral interpretations are made in earnest.

Let's get a quick preliminary read on candidates in IR for Tubes 3-9: (3-7, 3-8 same)

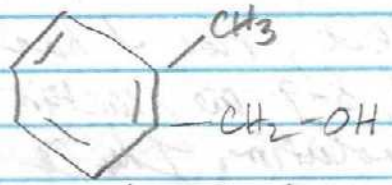
Absorbance in C:

		(3300-3500)	3060-3500
*	3329 strong	NH, amine	NH, amide or amine
*	3231 strong	amine salt (3145-3355) med	
		polymeric OH (3400-3225) (strong & broad)	
	3024 weak	C-H (of epoxide?)	C=C alkene (3010-3040)
		C-H of aromatic ring (3030-3015)	
*	1606 very strong	Carbonyl group C=O 1600-1820	
*	1458 strong	CH_3 (1460)	
	1352 weak	1340 C-H (weak)	
	1214 weak	Secondary amide (1200-1305) amide III (med)	
*	1029 moderate	S=O (1020-1050) strong	
		C-O methylene acetal (~1040) strong	
	950 weak	C-H vinyl ether	Vinyl ester (948) C-H
		N-O oxime	
*	656 very strong	C-H ~690 cis RHC=CHR' cis	
		C-S (570-705) thiol sulfide	

Let's look @ SDBS before we get too involved.
Strong to moderate peaks used in general
3329 3231 3024 1606 1458 1028 656
(+/- 15nm, T<70%) 15 matches
(+/- 15nm, T<60%) 7 matches

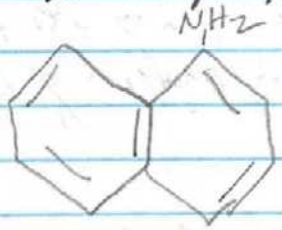
Recall what is seen w/ the NIR data also: ArCH, ArOH

Our closest match is methyl benzyl alcohol which is actually pretty decent. I bet no amine is listed and everything says we have an amine.

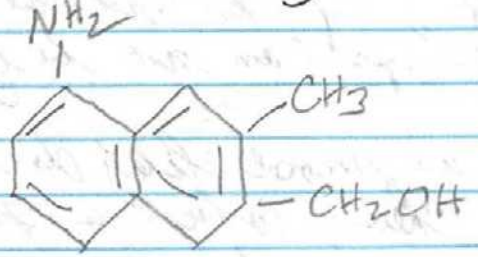


methyl benzyl alcohol

Second match is a naphthyl amine



Now, the combination of these two does seem very reasonable, ie, something to the effect of:



So our compound name here are naptho amine methyl benzyl alcohol

Naphthylamine is definitely showing up as a carcinogen, and is known to cause cancer of the bladder.

It causes genetic damage and mutations.

That particular compound is definitely bad news.

Methyl benzyl alcohol is considered to be slightly toxic.

Here is another observation on the protein colorimetric test, that I have seen before. Tubes 3-7, 3-8, 3-9 are reacting w/ the dye-reagent solution. They were noticed after sitting for 24 hrs. The tube contents are remained in solution homogeneous w/ the dye. Unlike as in the control the reddish dye settles to the bottom of the tube over time and there is a separation of color that take place.

We do have the emitted UV pass on these tubes. We also do have some conflicting signals here.

The carbonyl group can not be ignored. The amine groups can not be ignored.

The way that we most likely do have an amide bond here, which is the foundation for a protein.

The supposition of a protein like must remain @ the forefront of the interpretation & assessment.

Another significant question, especially in relation to both NIR and SOBS data, is the question of an aromatic structure.

Let's reexamine the aromatic absorptions.

NIR says it's there, albeit very weak. It says both ArCH & ArOH.

In addition, we noticed that a film does form on the ATR plate. This strongly suggests a polymer. We also notice that polymeric OH does show up in the IR candidates, alongside an amine salt candidate.

Ok, here is what we have from Koji: p 26-27:

"The presence of an aromatic group is shown by
(1) the 3030 [3024 cm^{-1}] and 1600-1500 cm^{-1} [1606] and substitution pattern is identified by strong absorption below 900 cm^{-1} [695]"

(2) "Condensed systems absorb @ 1650-1600"

(3) "1600, 1525-1450 (two) cm^{-1}; pyridines similar [1606] [1458] to phenyl group"

We have a lot of matches taking place here.

Notice also on May 29, 2019 when evaluating by IR tube 3-3 we also introduced

~~pyridines~~ pyrimidines (for the first time)

VS

pyridines (here.)

Is there a difference? What are they?

Pyridine Functional Group

Pyridine is a basic heterocyclic organic compound w/ the chemical formula C_5H_5N. It is structurally related to benzene = w/ one methine group replaced by a nitrogen atom. It is a highly flammable, weakly alkaline, water-soluble liquid w/ a distinctive, unpleasant fish like smell.

It has a role as an environmental contaminant.

There are important organic compounds in the literature of biology, environment and pharmaceuticals.

Pyrimidine is different. ~~It is a substituted~~

~~Pyrimi~~ Pyrimidine is an aromatic heterocy
SIMILAR TO pyridine.

One of the three diazines, it has the nitrogen at positions 1 & 3 in the ring. Formula is $C_4H_4N_2$

The other diazines are pyrazine and pyridazine.

~~Pyrim~~ Pyrimidine is one of two classes of heterocyclic nitrogenous bases found in the nucleic acids DNA & RNA.

So basically, pyrimidines are a parent component of DNA.

* We have fairly strong evidence that are separations involve both pyridines and pyrimidines, or related compounds

Now in Koji we also have additional comments on the absorption below 900 cm^{-1}. An increase in substitution on the aromatic ring is shifting the absorption further below 900 cm^{-1}.

Five adjacent H atoms shifts the absorption down in the range of 690 cm^{-1} to 770 cm^{-1}, much closer to what we see.

Tubes 3-7, 3-8, 3-9 Summary Isolated LC Separation: Important Compound ~~Identified~~ Here.

Our summary thus far indicates that, now as that a distinct pattern that we have the following functional groups or structures / recently increased attention.

	1. Amines	With an overriding interest in DNA benefit not all.
*	2. Amides	
	3. Pyridines	
*	4. Pyrimidines	
	5. Polymeric development	
	6. ArCH & ArOH Candidates	
	7. Aromatics & derivatives	

* Notice that DNA reference, building blocks and constituents appear to keep consistency in the analysis of both the polyhalid and H_2O insoluble CDB culture growth.

Continuing, now @ Tube 3-10

Previous Ref.	X	7.38	7.04	84	1.7	
	t	EC	pH	ORP	Flow Detector	Solvent
Tube 3-10	1830	9.23	6.98	+109	14/3 = 4.7	HCl

Tube 3-10 is reasonably consistent w/ Tube 3-9.

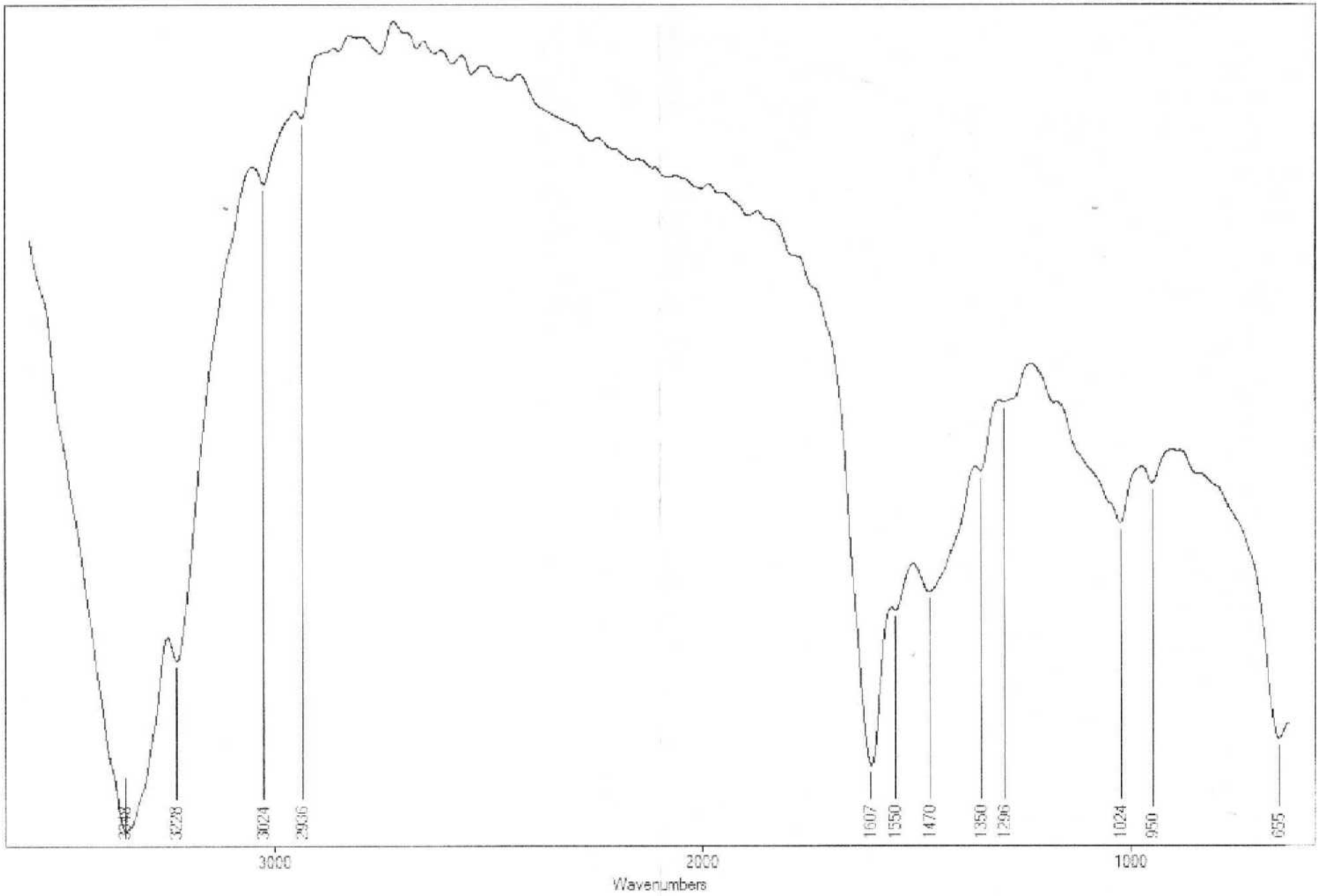
There is a definite thin film layer that forms on the ~~and~~ plate. A polymer form is developed there

Tube 3-10 - Fundamentally the same as 3-8, 3-9

CDI

prin

CDB Solid Protein - LC - Tube 3-10 - HCl Solvent - May 31 2019 - 17.spc: 05/31/2019 19:57:16 title



printed on 05/31/2019 20:05:41

Tube 3-11	t	EC	pH	ORP	Flow Det.	Solvent
	2115	7.05	7.44	138	14/3 = 4.1	HCl

No change expected here.

The column appear stable @ the point of the run w/ HCl will be terminated upon emptying the reservoir.

The next solvent used will be that of ammonia but it will be diluted by ½ ~~1/4~~ w/ H2O 3/4 due to previous experience, precipitation & subsequent plugging of the column.

Tube 3-12	2140	NA	10.36	-46	24/2 = 12.0	Dilute Ammonia

There is indeed a precipitate that is forming already here and the column is slowing down. Small sample collected.

Tube 3-13	~~2.10~~ 2150	2.10	11.83	-75	26/1 = 26.0	Dilute Ammonia

Notice how the flow detection indicator has easily shown the change in the column due to the ammonia

Tube 3-14	2205	1.95	11.73	-50	16/1 = 16	Dil. Ammonia

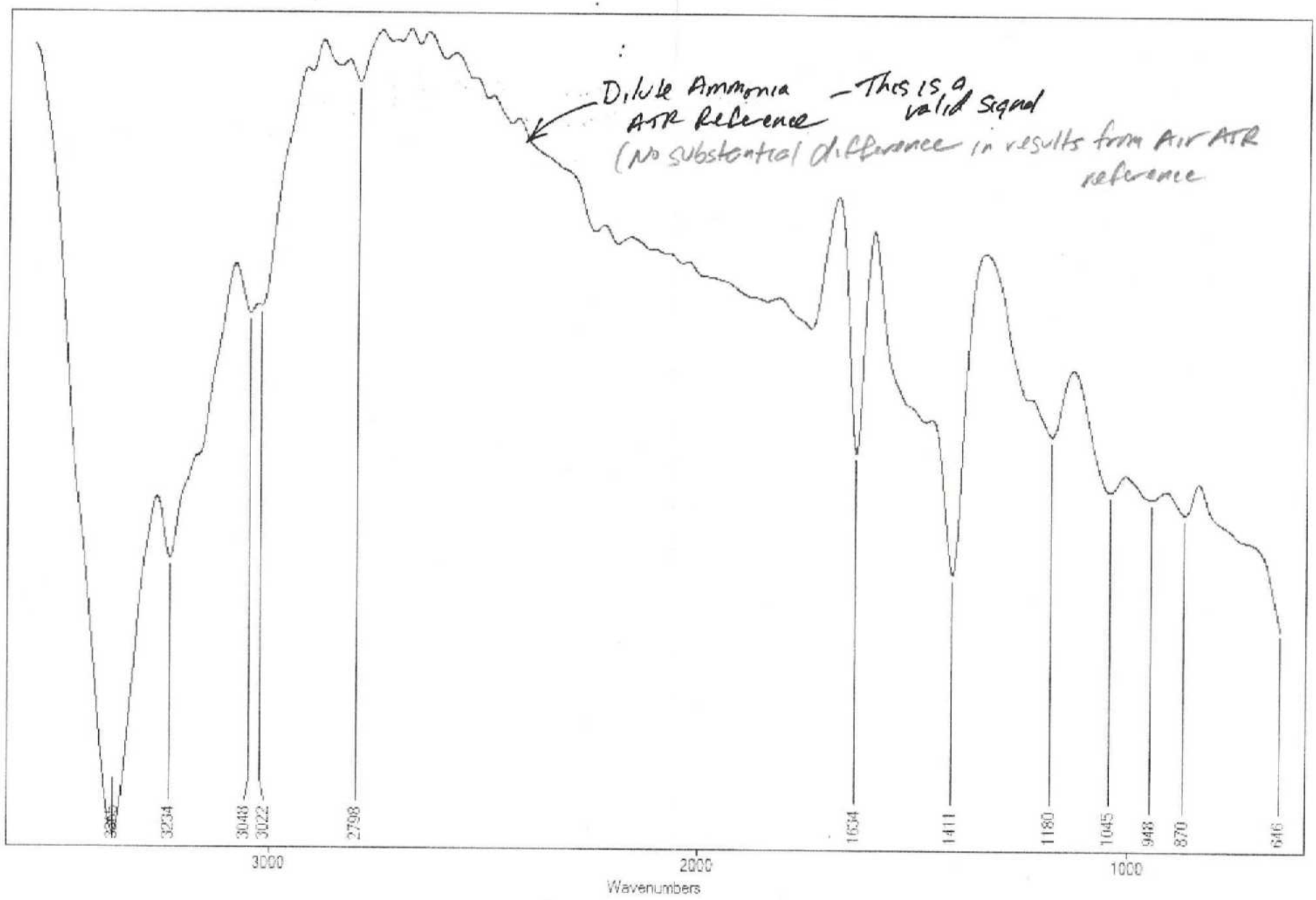
Now, the pH of Tube # 3-12 is higher than is preferred for the ATR plate. Use caution here.

Tube 3-12 Ammonia Solvent Subtracted - Valid Signal Here

CDB Soli

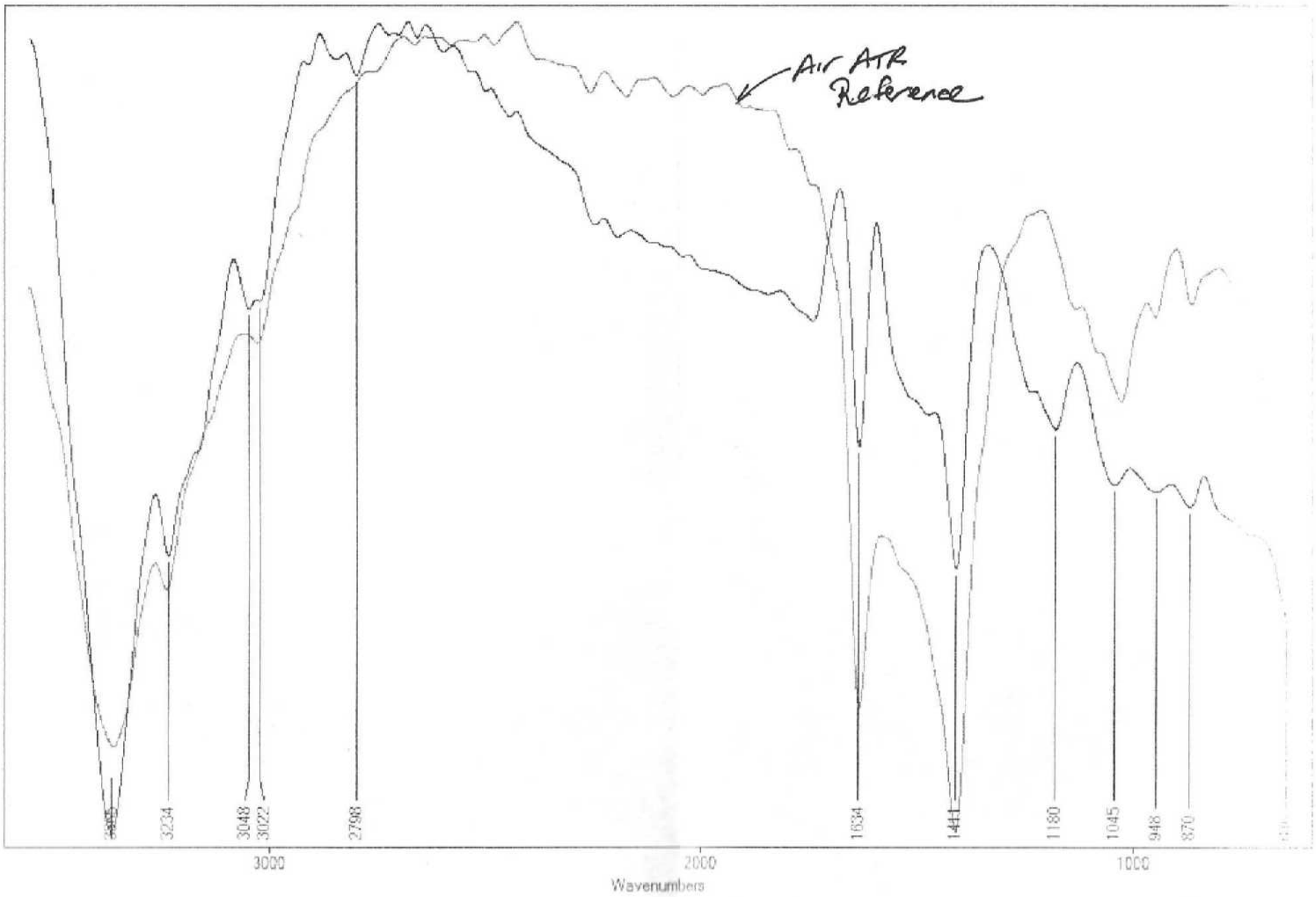
printed o

CDB Solid Protein - LC - Tube 3-12 - Dilute Ammonia ATR Reference Subtracted - May 31 2019 - 19.spc: 05/31/2019 23:53:02 title



printed on 06/01/2019 00:00:14

CDB Solid Protein - LC - Tube 3-12 - Dilute Ammonia ATR Reference Subtracted - May 31 2019 - 19.spc: 05/31/2019 23:53:02 title



printed on 06/01/2019 00:01:00

Since we know that ammonia has NH in it, we must run an ammonia reference spectrum on the ATR prior to any interpretation of Tube 3-12.

The column is now darker (tan color) than before w/all runs. The data indeed indicates increased precipitation of the column has occurred, most likely an iron compound.

Definitely a strong ammonia odor in each Tube of the ammonia run.

to Subtracting the ammonia solvent influence was highly successful.
Findings is that our signal is valid above and beyond any ammonia influence.

Tube 3-12 data requires analysis. A compound has been separated & extracted.

Tubes 3-13 & 3-14 can be held as additional references if required.

The column eluate remains stable.

The question now before us is how to remove the remaining tan precipitation in the columns? The good news is that @ this point there is no sign of the columns stalling out, however you must remain cautious w/ any base acting as a solvent.

Let us switch back to vinegar to see the effect.

There is nothing known yet to completely dissolve all precipitation that also does not create the risk of destroying the columns.

	t	EC	pH	ORP	Flow Det.	Solvent
Tube 3-15	2320	3.90	4.60	315	38/2 = 19	Vinegar
	A definite clear film layer formed on ATR plate					
3-16	2335	2.57	4.37	324	24/2 = 12	Vinegar

On May 26, 2019 we included a reference SDBS spectrum of acetic acid. It did that for Tube 3-15 w/ the switch to vinegar that one must do the same thing we did w/ ammonia, ie, create a reference ATR spectrum w/ vinegar (acetic acid) as the background in order to subtract it from Tube 3-15.

In addition of Can see that strong HCl is not destroying the columns as it has now been packed, but it does effect it. It definitely reduces the gravel caps to a fine powder. There is very limited use of any one column. Some fine sand is to be replaced. I will try HCl after the 2nd range run.

Tubes 3-11

&	EC	pH	ORP	Flow Detect	Solvent
					Strong HCl

The column is most definitely turning white in the upper half. It is also almost plugged up but fortunately not quite. Definitely a last fraction for the column. Flow rate is now: 4/3 = 1.33

The effort to settle the reference spectrum for acetic acid (vinegar) was a well moved. We see that the reference spectrum for acetic acid and Tubes 3-15 & 3-16 are essentially identical.

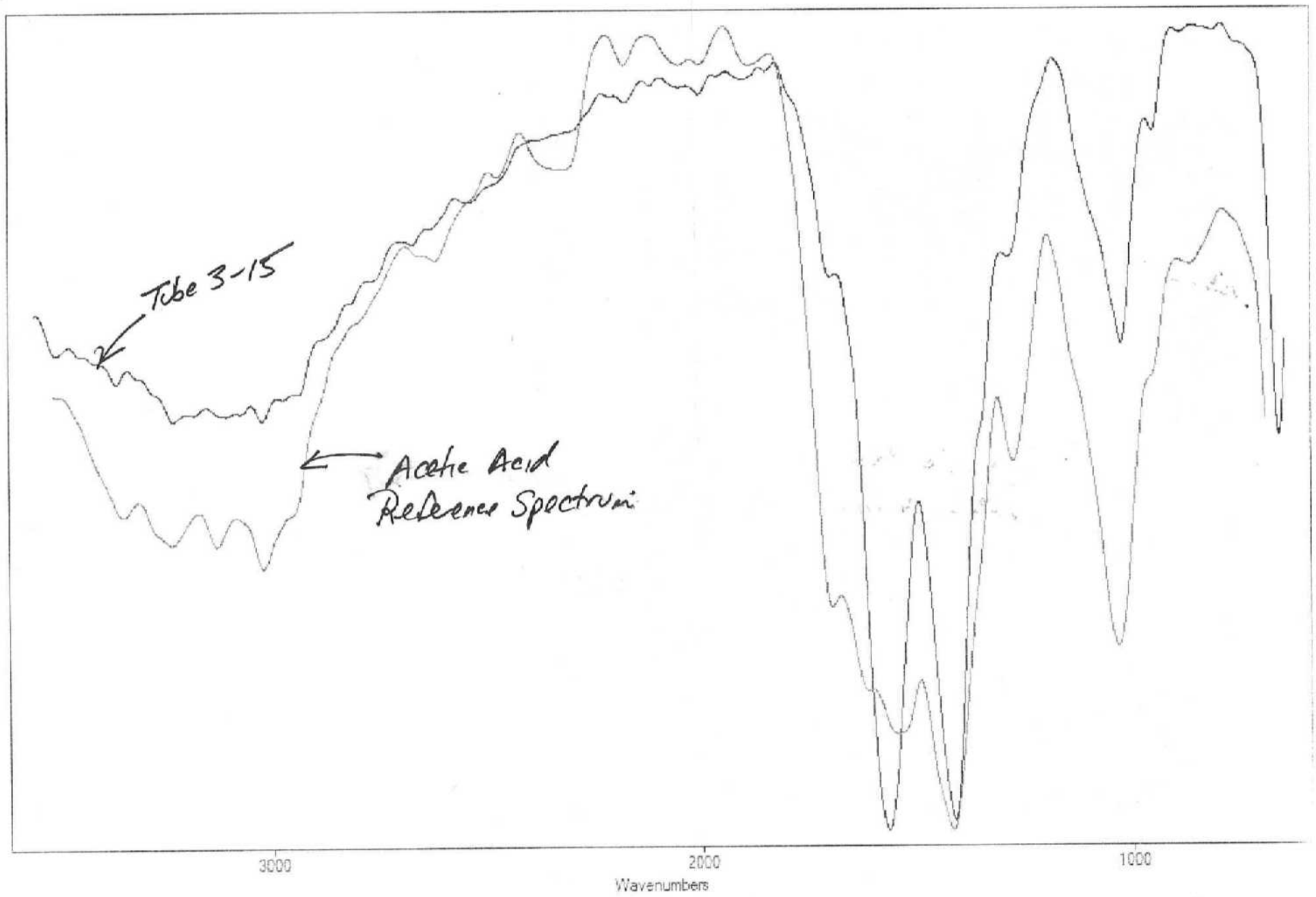
The only compound that came through on Tubes 3-15 & 3-16 is acetic acid.

Disregard these two columns.

Reference spectrum of Acetic Acid - Tube 3-15 Comparison

printed on 06/01

Vinegar - Acetic Acid - Reference Spectrum - May 31 2019 - 20.spc: 06/01/2019 01:38:15 title



printed on 06/01/2019 01:39:53

What happens w/ the column along w/ all the radical activity of using strong HCl is that the upper 1/2 of the column is now pure white, which certainly looks to be an improvement.

However, the lower 1/2 of the column remains static & tan in color, and there still is no assurance that the precipitates or solid forms will still dissolve or migrate through the column.

The flow rate remains slow but it is gradually improving. This is also a good sign. The last column that was subjected to strong HCl has held up well. The packing of the column, especially the lower end cap (glass beads, small glass beads, coarse sand) appear to be critical to the success or else we know that the column is destroyed.

Flow Detector indicator is now @: 6/3 = 2 vs 1.33

I have added more HCl to see if any of the tan color can be pushed through the column.

Flow rate is now: 4/3 = 1.33

3-17 0145 >20 4.59 +271 5/2 = 2.5 Strong HCl

June 02 2019

An extremely important consolidation of the research has taken place over the last couple of days.

The final, tan colored, compound in the LC column has presented numerous challenges to extraction and separation. The residual material derives the solid growth of the CDB cultures. As we recall, major advances in the cultivery methods were made at the close of last year.

The most radical method being used so far in the attempt to remove this precipitated material in the column is w/ the use of very strong hydrochloric acid.

Strong HCl is very difficult to manage in the column but I have learned to do so with proper packing of the column.

It remains a borderline difficult mixture, but I now see, through IR analysis,

that I have been able to extract a small portion of the residual material.

It is contained within Tube 3-17 of the work on May 31 2019.

A solid IR spectrum has been obtained. This requires specialized method of gradual evaporation of the sample upon the IR ATR plate that I have developed. All samples are of low concentration relative to the solvents that are liberty used.

In this case the solvent is concentrated HCl.

HERE IS WHAT IS FOUND:

In matching this IR spectrum to any library entry or previous IR work done,

THE BEST MATCH (OF ~7000 spectra searched)

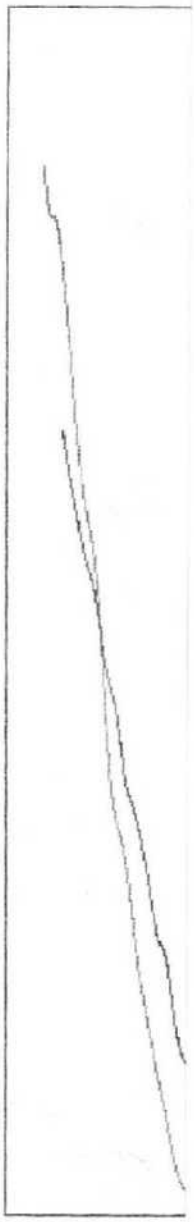
* BELONGS TO THE 1999 ENVIRONMENTAL
* FILAMENT SAMPLE (THE INFAMOUS EPA
* "ENVIRONMENTAL FILAMENT") that was finally successfully digested by microwave-solvent methods in 2017.

This completes an important and complex loop of the research that has now taken place for more than two decades.

An Important Achievement

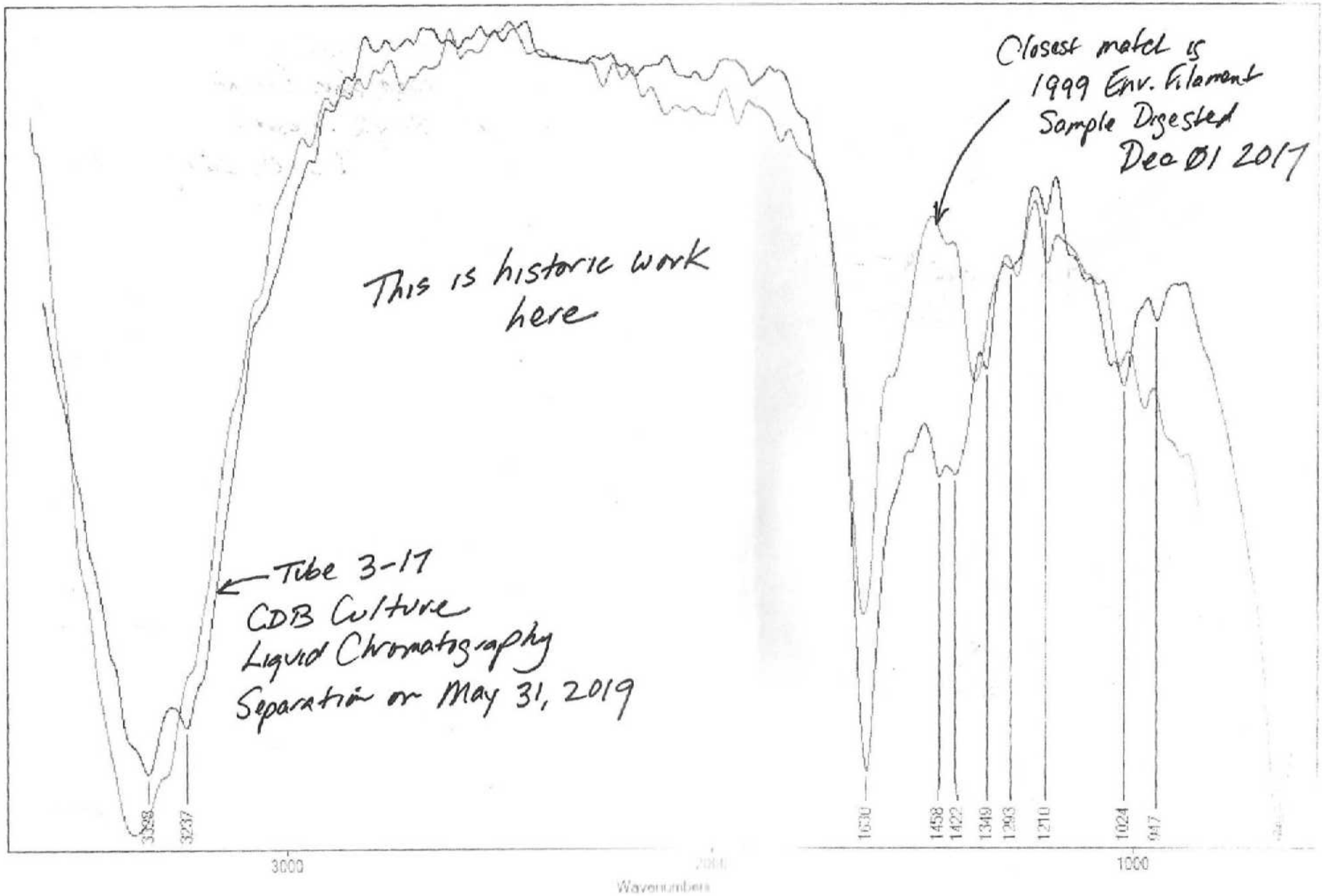
* is Recorded Here June 02, 2019

CDB Solid Protein - LC - Tub

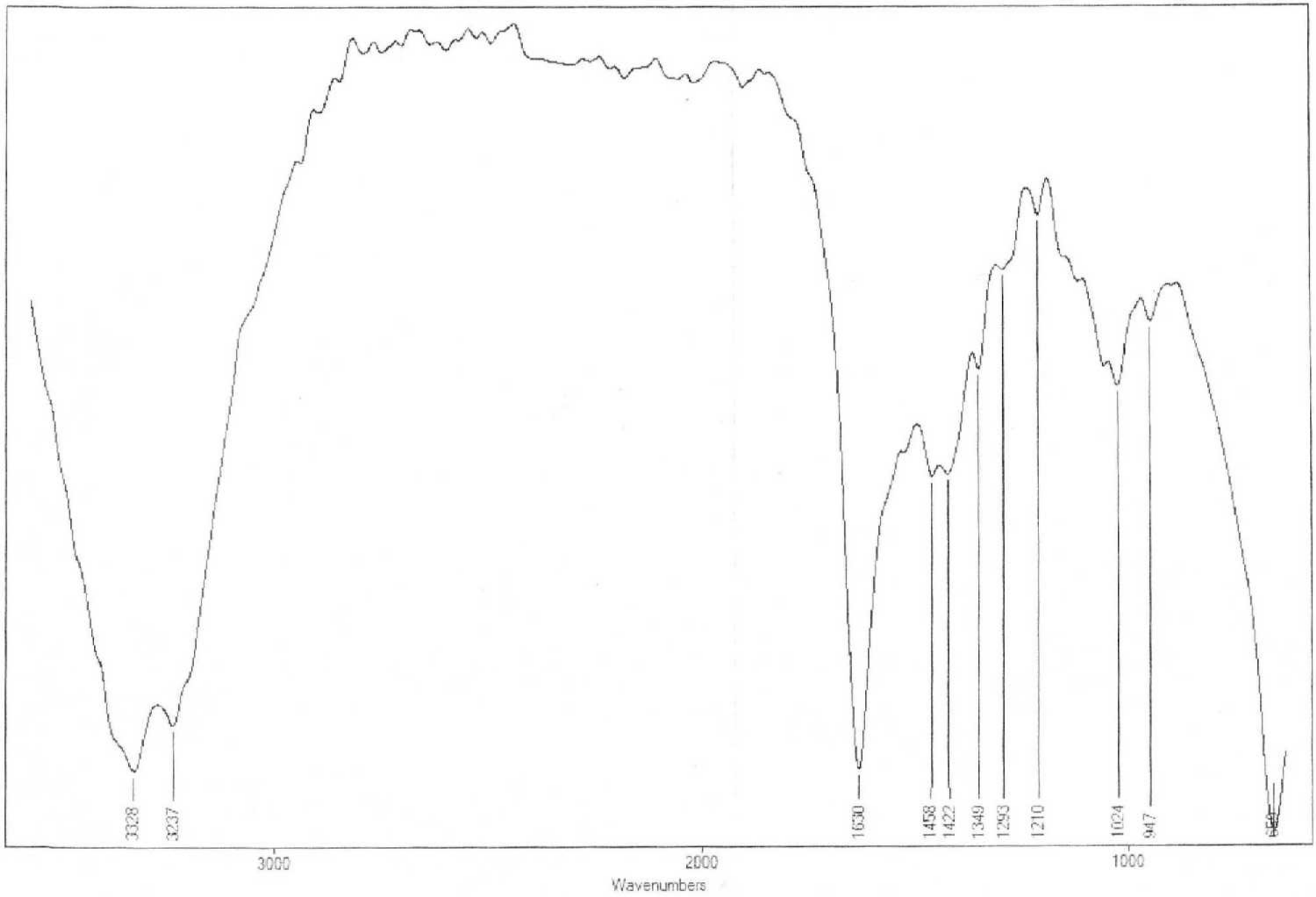


printed on 06/01/2019 03:20

CDB Solid Protein - LC - Tube 3-17 - Strong HCl Solvent - May 31 2019 - 21.spc 06/01/2019 03:13:27 title



CDB Solid Protein - LC - Tube 3-17 - Strong HCl Solvent - May 31 2019 - 21.spc: 06/01/2019 03:13:27 title



printed on 06/01/2019 03:20:07

Historic Accomplishments of the Research are Stated Here,

The work demonstrates the direct connection & equivalencies of

1. The infamous "EPA Environmental Filament" event(s)

2. The structural, chemical & molecular analyses of the same "environmental filaments" w/ limited test methods & technology over the two decade period, but nevertheless providing unique, complex and repeatable data & information

3. A series of advanced microbial culturing methods, dependent upon that same "environmental filament" source material.

4. The structural, chemical & molecular analysis of those same microbial metabolic products from that same culture.

5. The demonstration of equivalency (structurally, chemically & molecularly) between the "environmental filament" and the products of cultures in a controlled environment.

A loop (and indictment) of great importance has been sealed here.

These parallel plots will need to be studied in great detail and a review of any notes for the Dec 2017 period made.

First impressions based upon the closest matching set of spectra may be the use of the keratin or keratin like compounds that seem to serve primarily the function of encasement of the microbe.

Our sample produces a highly limited in concentration & volume.

I will work further to see if any additional material can be extracted from the column.

Reference LC values are

3-17

EC	pH	ORP	Flow Det.	Solvent
720	4.59	+211	2.5	Strong HCl

Notice the high conductivity that appeared here. Let us test the HCl solvent conductivity: It is also ≥ 20 The high conductivity, therefore, does not result from the compound so much as it comes from the HCl. Nevertheless, the high conductivity permits the separation to take place, at least to some degree.

I will now increase the solvent to be that of H_2SO_4.

	t	EC	pH	ORP	Flow Dir	Solvent
Tube 3-10	1420	2.13	3.94	500	Rapid (22/1) = 22	H_2SO_4

The Lac worked. I am now getting color from the LC tube.

I only have 3-4 ml of the extract but I most certainly do have it. Within the H_2SO_4 it is a fairly bold yellow color

The Column is perfectly white now. Time to flush out w/ H_2O. This is the most extreme solvent that I could ever expect the LC Column to be subjected to, and it has survived w/ out any damage. This is entirely attributed to the packing method of a Column that has been developed.

You really do not need any Coarse sand @ the top of the Column any more. Fine sand is sufficient.

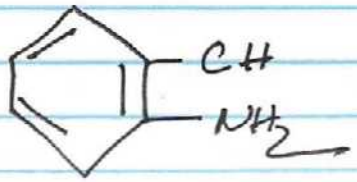
The Column is slow now w/ H_2O as the solvent, but is still working fine.

With NIR analysis of Tube 3-18, we have definite absorbance @ ~ 1029 nm which corresponds well to

RNH_2 No real sugars here. Also no competition here.

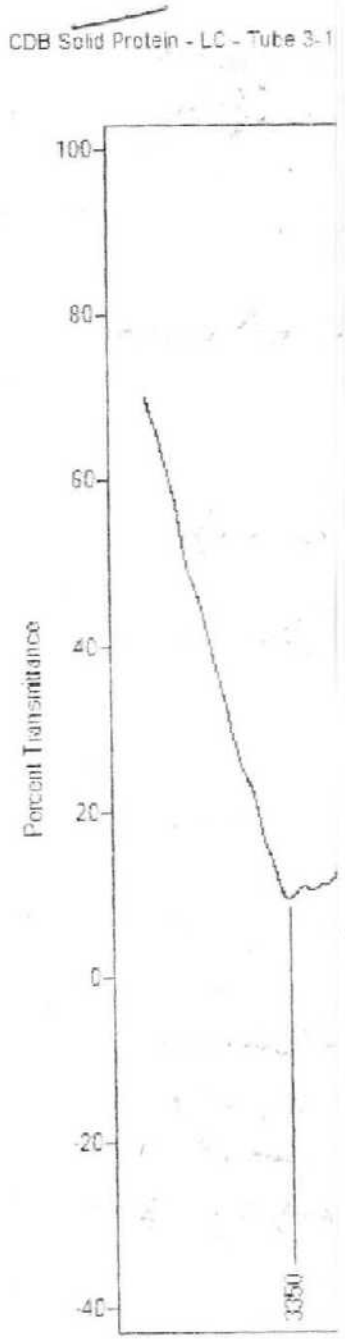
We also have absorbance @ 762 nm. This too corresponds to RNH_2.

We also have absorbance @ ~ 862 nm. This corresponds to ArCH.

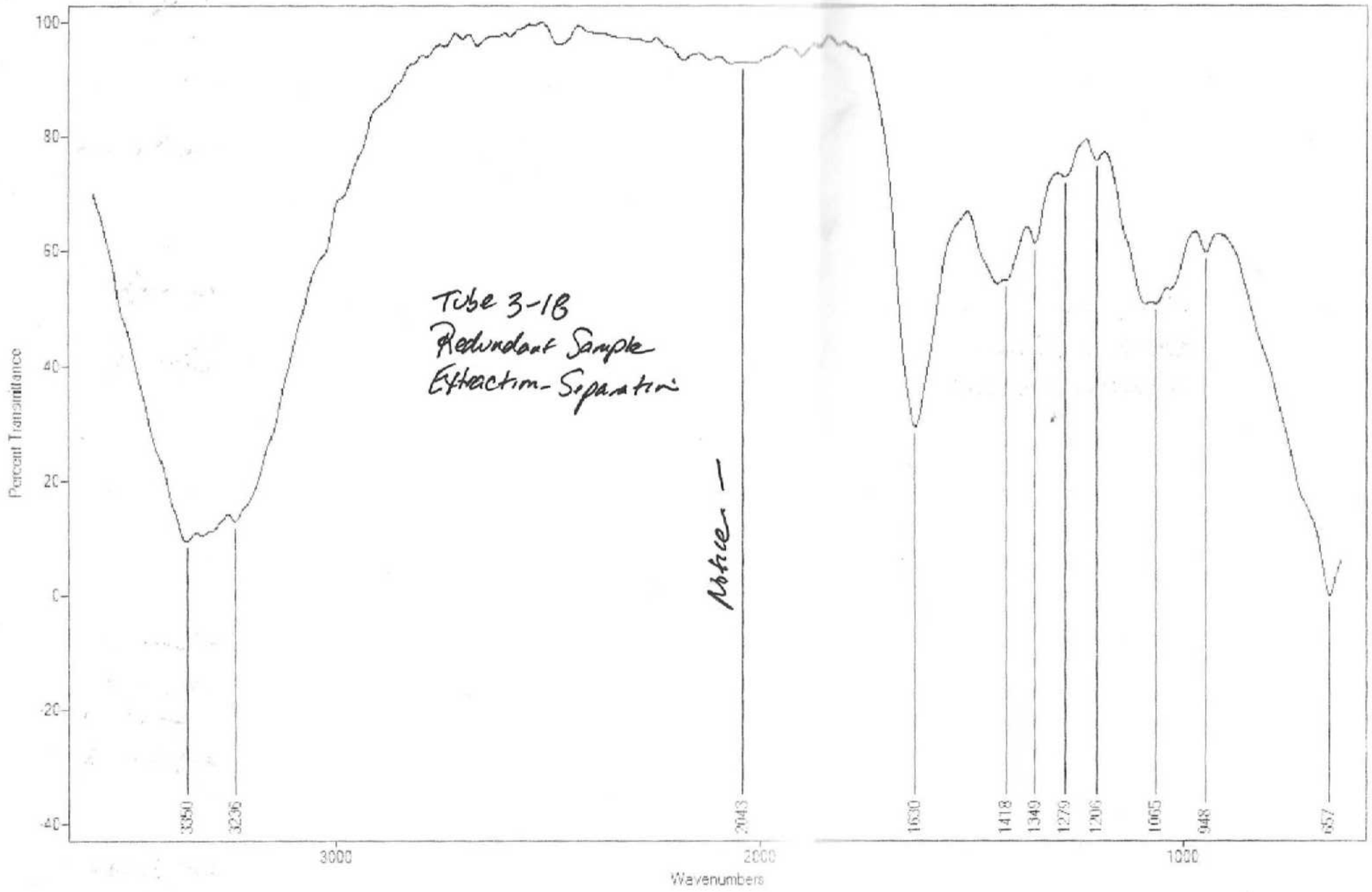
This says  as a minimum.

A good candidate for 1. protein colorimetric test
2. amine test
3. Electrochemistry - Inorganic

3-17 & 3-18 "
Tubes ~~3-18 & 3-19~~ vs Environmental Filament"
Comparison Spectra

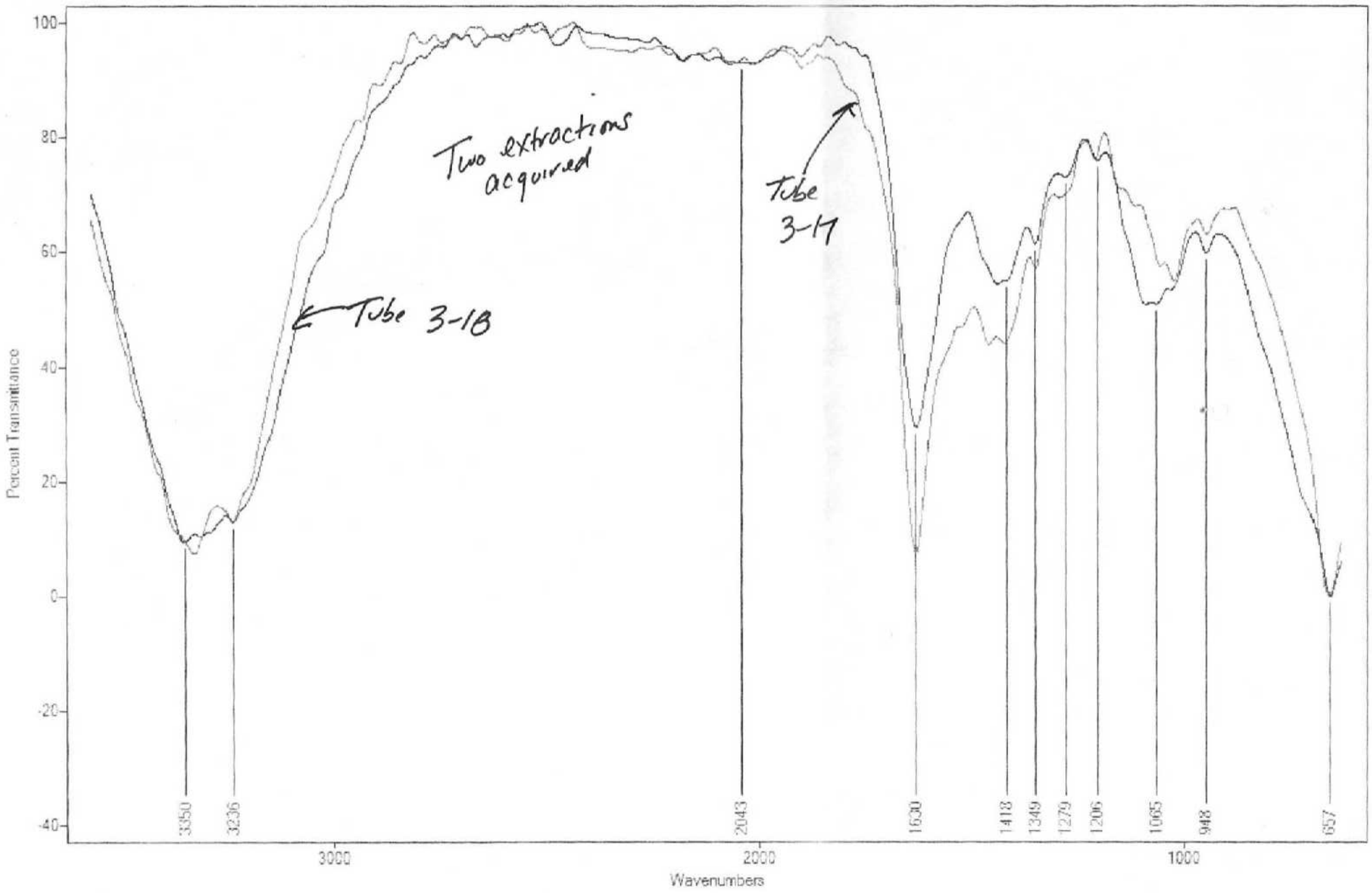


CDB Solid Protein - LC - Tube 3-18 - Strong H2SO4 Solvent - pH Neutralized - Jun 02 2019 - 22.spc: 06/02/2019 16:09:35



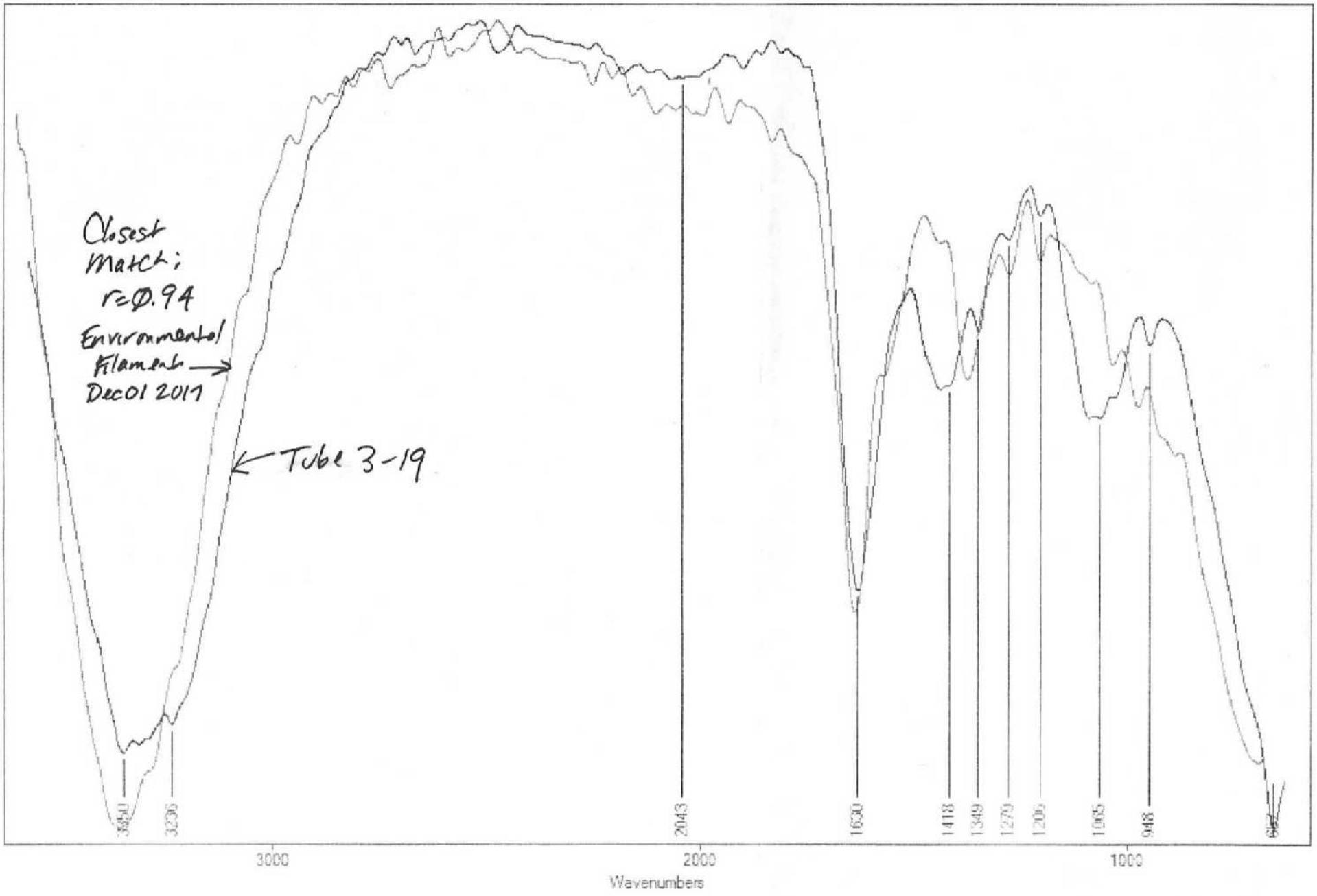
printed on 06/02/2019 16:22:10

CDB Solid Protein - LC - Tube 3-18 - Strong H2SO4 Solvent - pH Neutralized - Jun 02 2019 - 22.spc: 06/02/2019 16:09:35 title



printed on 06/02/2019 16:22:29

CDB Solid Protein - LC - Tube 3-18 - Strong H2SO4 Solvent - pH Neutralized - Jun 02 2019 - 22 spc: 06/02/2019 16:09:35 title



printed on 06/02/2019 16:26:02

Additional Important Statements of Fact:

I now have 2 samples of the final extract & separation from the CDB LC Columns.

They are essentially identical although ~~3-19~~ 3-18 is more dense in color than ~~3-18~~ 3-17 is. ~~3-19~~ 3-18 evaporates to a fairly dense film on the IR ATR plate & a good signal is attained.

We also know that ~~3-18~~ 3-17 & 3-18 have the closest match as the digestion of the "Environmental Filament".

* The claim of the final products of culturing the CDB microbes as matching the source microbiology of the so-called "Environmental Filament" (confirmed by the EPA)

* IS NOW COMPLETELY & TOTALLY CONFIRMED.

Observation: Some change has occurred in the protein colorimetric test whereby it is less sensitive than before and as necessary. I suspect it has to do w/ pre-diluting the dye and placing it into storage.

All negative results results in recent protein colorimetric tests (last 3-5 days) must be disregarded, as false negatives are likely to have occurred.

Let's look back @ our dilution ratio.

500 ul red dye in 30 ml
or
200 ul red dye in 12 ml H_2O.

And the answer is YES. The dye solution MUST BE MIXED FRESH.

else the sensitivity is lost, which has been somewhat of a shock of mine over the last several days.

I think we may have missed some positive confirmations for protein existence. I will retrace those steps.

200 ul Ritz Red Dye in 12 ml H_2O is appropriate.

Protein Colorimetric Formula is

1. 3 ml H_2O
2. 1 drop 10M NaOH
3. 2 drops 0.5M $CuSO_4$
4. 100 ul Diluted Red Ritz Dye
   (200 ul Red Dye in 12 ml H_2O)
5. Pinch Cream of Tartar

Every protein Colorimetric test must have a control tube.

3-18

Now after the passage we see that Tube 3-19 is actually composed of a solid that settles. It is pure white after exposure to the concentrated sulfuric acid.

I have ~ 1-2 ml of white solids that has settled in the tube. This is undoubtedly a valuable sample that has been very difficult to extract & separate.

Tube 3-17 passes the protein colorimetric test w/ flying colors

---

Even Tube 3-17, mostly dissolved if not all, in highly concentrated acid produces a vigorous purple color change in the test.

There is a concentrated protein in Tubes 3-17 & 3-18 and Tube 3-18 consists primarily of solids.

The protein must be slightly soluble in concentrated acids.

The Ninhydrin test for Tube 3-17 is negative.

Therefore, we have AMIDES, not amines,

i.e., proteins

Repeat of Pre Summary Analyses, here for kicks, & am running a ninhydrin test on ammonia
Ammonia Comes out BOLD YELLOW. So not everything w/ Ninhydrin is purple. We did have a definite reaction.

What must now be done is to repeat our colorimetric test for proteins on each of the recent LC separations of the CDB solids.

OK✓ Tube 3-1 Protein Result is negative.
There are solids settled @ the bottom of the tube. Only the solution (clear) has been tested.

Let's look @ original results.
The original notes give a negative result.

I think it would also be wise to collect NIR data on each of these tubes.

NIR gives absorbance @:
~1012 nm RNH_2
~912 nm CH_2 or ROH

There is an interesting result @ ~1012 nm.

Ninhydrin test is negative, always conditional upon concentration.

Protein test also dependent upon concentration.

~~Tube 3-2~~: Notice that we do have some conflicting NIR data for Tube 3-1. Notice also that we examined the NIR database. Notice also that we investigated both the solid & solution forms in the tube. The tube requires further examination & reconciliation.

Notice that WE DID identify an amine previously for Tube 3-1. The very most large the question of concentration. Let's repeat the test - this time I will shake the tube.

I have reduced the H_2O to ½ (~1½ml), 600ml of Tube 3-1, & 10 drops Ninhydrin, ie, I have attempted to increase the concentration level.

This is a great example of when concentration makes a big difference. We are almost always dealing w/ minimum detectable concentrations in our samples. This can occur for both the ninhydrin and the protein Colorimetric tests, so a negative Colorimetric test does not necessarily mean the absence of either an amine or a protein.

WE DO NOW INDEED HAVE A REACTION. Our Color is actually a orange-purple but it is a desperate Colored & purple reaction. It took the above changes, increased heat & increase in time to develop the reaction to the point of becoming visible.

Our analysis of prior Tube 3-1 remains perfectly valid.

It is assessed, as a mnemonic, as being an "Aromatic polar amine Compound."

Tube 3-2 Presumably the same as 3-1 ?

Tube 3-3

The tube brought up the existence and/or question of pyrimidines

Stop the Press!

As we know, it is best to never assume. Let's look @ Tube 3-2

First: NIR:

We see that the concentration is very weak (full strength Tube 3-2 used) limit detectable absorbance @ ~1010 nm & ~900 nm.

1010 → RNH_2

900 → Most likely CH_2 or CH_3.

Second: We see that the protein colorimetric fails

Third: We see that the Ninhydrin test is POSITIVE exactly as in the same way as Tube 3-1.

Now, and only now (:) can we proceed to Tube 3-3.

Tube 3-3 is a very small sample so we must be careful. Let's look @ prior information:

Tube 3-3 has defenite NIR absorbance @:

~1012nm RNH_2
~912-915nm CH_2 or ROH
(NIR only!)

NIR: Notice that this is identical to Tube 3-1. Sample material is scarce @ concentration concerns exist as always. I recommend an amine test next.

Notice our IR plot of 3-3 is very weak w.r.t. amine detection.

Our colorimetric test is developed as follows:

Visible detection by eye of an amine-ninhydrin reaction is borderline. Therefore I look towards to VIS spectroscopy between 380-800nm.

In fact, I do pick up a slight absorbance peak @ ~537nm. This means we have absorption in the green range and to the eye this then appears as a red-purple.

Conclusion: We do indeed have an amine reaction
* Positive Test Result here.

Next to Tube 3-6. Those w/ Vinegar.
Apparently all passed an amine test.
Let's see. NIR comes first

Absorbance take place @
1065 cm^{-1} some stance of RNH_2; mixed stance of ArCH
894 cm^{-1} ArCH

Most likely candidates w/ NIR seem to be both RNH_2 & ArCH.

Protein
Protein

Here we go. Tube 3-6 absolutely & quickly is positive on the protein colorimetric test.

There is no sign whatsoever of an amine test being positive in this analysis.
I have no idea where that came from.

The protein test, however, is quite positive.

Now for Tube 3-8:

Protein Test & NIR are important here.

Protein Test is negative; matches previous result.
Amine test is also negative; also matches previous result

NIR shows some absorbance @ ~ 1066 nm
Closest match here is Ar-CH, but it is not exactly in range.

Notice that IR plot is a strong signal, however

Interesting previous notes. Seems to have conflicts between possible NH presence in IR but failure on host colorimetric tests as well as NIR showing no NH or NH_2.
SDBS was also used as a reference.
Most
~~Everything~~ says no problem, no amine? But? Concentration levels? Polymeric OH?

3-1 # 3-10
Tubes ~~3-4~~, 3-8, 3-9 received some extensive IR analysis

Notice 3-1 thru 3-11 are w/ HCl.

We have a rasha developed summary of analyses for Tubes 3-1 to 3-11. SDBS was undoubtedly a primary tool here

There remains a series of questions about these series. Appear to be a unique & important compound but not problem

Next is the Combined series of Tubes 3-12 thru 3-14.

We have a strong signal here independent of ammonia but nevertheless realize that ammonia was used as the solvent.

We ran one Control on IR with ammonia and also we ran a methyl chloride test w/ ammonia. We must keep these in mind. We also need NIR on ammonia.

Ammonia NIR: The result here was inconsequential with one important exception.

First of, the chemical formula for ammonia is?

Pure ammonia (NH_3 is a gas) called Anhydrous ammonia.

The gas dissolves in water to form NH_4OH and it is called ammonium hydroxide.

What happened w/ NIR is that I recalibrated before making the run. The run gives a single absorption peak @ 960 nm.

This corresponds to H_2O. Without much doubt The closest competitor is ArOH and we know that we do not have this w/ ammonia.

Therefore there is a reason here w/ the 960 nm absorption. We must treat it carefully and not assume H_2O is irrelevant. In the Case water & the 960 nm does absorption seem especially important.

Now given 960 nm it's due recognition (water out to previous errors) then the NIR plot is essentially flat w/ no NH peaks recognized. (Because we are actually dealing w/ NH_4).

The ninhydrin test is also important. We get yellow instead of purple. No papers seem to be talking about this.

So now, what do we find in Tube 3-12?

With NIR, we get exactly the same spectrum as for ammonium hydroxide. The indicate no additional NIR absorption is to be found in Tube 3-12.

We also see that Tube 3-12 fails the ninhydrin colorimetric test.

We also see that Tube 3-12 does have a reaction w/ ninhydrin (somewhat similar to that of ammonia turning yellow) but it is a darker a bolder yellow.

We see that, from research, ninhydrin does react w/ numerous compounds besides just amines, such as amine salts for example, w/ a variety of colors.

We would say @ the point that we do have a presence of a culture for Tube 3-12 as

1. a Protein
2. as an amine
3. as long NIR absorption.

And yet we know that we do have a significant signal in IR so we will refer to digital specs that interpretation for primary importance.

Lastly we go to Tubes 3-17 & 3-18 which are of recent analysis and known to be proteins.

Next we will summarize results.

June 04 2019

I am working w/ a semi-automatic titrator now. I am getting things to work nicely now; it has taken some work.

Two major advantages to the system compared to my drop counter method from before.

1. Dispensing the titrant is now automatic, at two different speeds.

2. The data is being recorded for the last 100 dispenses of titrant.
3. Magnetic Stirring is perfectly quiet - great.

A couple of unexpected requirements needed to be met:

1. The titrant must be gravity fed as you needed to develop a graduated burette system that is not too tall. A 60 ml syringe w/ the plunger removed fits the bill. [12 ml pipette is even better]
2. Although dispensing of titrant is automatic by time, knowledge of volume is not a part of the data recorded. The answer is to calibrate the loss of volume in the syringe in accordance w/ the recorded count.

I believe I have a working system.

Calibration of volume:

1st Trial Fast speed: $\frac{21\,ml}{52\,counts} = \frac{X}{1}$ $X = 0.404\,\frac{ml}{count}$

The gravity tube must be primed to get an accurate read

#2 Fast: $\frac{33\,ml}{90\,count} = \frac{X}{1}$ $X = 0.367\,ml$

→ You actually do not need to worry about this; the solenoid draws the fluid evenly.

#3 Fast: $\frac{34\,ml}{94\,count} = \frac{X}{1}$ $X = 0.362\,ml$

#4 Fast: $\frac{35\,ml}{96\,count} = \frac{X}{1}$ $X = 0.365$

Titration is limited to a count of 100.

$\bar{X} = (.367 + .362 + .365)/3 = 0.364\,ml/count$

Quite reliable it seems.

Next we look @ the slow speed. I am using a pipette as the reservoir of a 12 ml capacity. This is indeed a fine calibration - looks excellent.

#1 Slow

$$\frac{4\text{ml}}{78\text{count}} = \frac{X}{1} \qquad X = .051\text{ml/count}.$$

This is excellent as it is equivalent to 1 drop.

#2 Slow: $\frac{4.1\text{ml}}{80\text{count}} = .051\text{ml/count}$

Very Stable result: Calibrated pipette = 0.051 ml/count

This means we now have a excellent calibration of both fast & slow speeds. It requires either a

1. 12 ml pipette for fine work
2. a 60 ml syringe adapted w/ tubing for general work.
3. A 150 ml syringe is coming in for coarse work.

1. This is actually very accurate most.

Now, how would we transition from general work to fine work?

a. Can you carry this on the road?

Let's try a trial titration w/ acetic acid and ammonium hydroxide (vinegar & ammonia).

Let's use 26 ml of acetic acid as the analyte. Let's use NH_4OH as the titrant.

First, flush the burette (12 ml pipette) w/ NH_4OH.

Use a syringe to fill the burette for fine dumps - control all liquids & spills

pH of analyte is 1.68

Set endpoint of titration to pH 10

Fast	Count = 8,	pH = 1.96	to	pH 2.0
Fast	Count = 29	pH 2.0	to	pH 2.56
	Refill burette.			
Fast	Count = 40	pH = 2.56	to	pH 2.91
"	Count = 42	pH 2.91	to	pH 3.13
	n = 41	pH 3.13	to	3.29
	n = 42	3.29	to	3.44
	n = 42	3.44	to	3.60
	n = 41	3.60	to	3.76
	n = 41	3.76	to	3.94

These results show that acetic acid is actually a very strong acid. Not a surprise by initial pH ≈ 2.0

Let's regroup on this and use 5 ml of acetic acid in a total of 25 ml titrant diluted w/ H_2O.

We have 5 gms (5 ml) acetic acid diluted to 25.1 ml H_2O.

Initial pH = 1.83 Fast mode

		~ Volume
n = 41	pH = 3.37	~12 ml
n = 41	pH = 4.10 4.42	~12 ml
n = 42	pH = 7.90	~12 ml
n = 40	pH = 9.77	

Most rapid pH change occurs between n = 23 & 24

n	pH
23	6.01
24	6.26

$\bar{x} = 23.5$ pH = 6.14

Volume = 41 + 41 + 23.5 = 105.5 (0.293 ml/count) (.364 ml/count) = 38.4 ml @ equivalence point. = 30.9 ml

30.9 ml

Check on Volume Calibration: $\frac{\sim 12 \text{ ml}}{41 \text{ Count}} = \frac{x}{1}$ $x = 0.293 \frac{\text{ml}}{\text{Count}}$

Ok, the stock in that volume needs to be calibrated for each setup & run. This is not difficult using a 12 ml pipette.

I think that a pipette will be used as the burette for almost all occasions

Ok, the lab worked perfectly.

Numerous advantages here:

1. No need to count drops or volume except coarsely
2. Easy to assess proper range of titrant / analyte
3. Data is recorded so equivalence point can be easily identified.
4. Magnetic stirrer is quiet.
5. The titration can be done in sequential stages, either in fast or slow mode per sequence.
6. Strong - weak acid / base easy to establish
7. Will be simple to do any chain titration from known acid / base to test an unknown titrant and an unknown analyte.
8. The process is relatively fast
9. Calibration of flow rate will be done for each partial setup (Hoffman clamp adjusted).

In the case, we see clearly that acetic acid is a strong acid and our ammonia is a weak base.

In our case 30.9 ml of commercial ammonia reacted with 5 ml of acetic acid diluted w/in a total volume of 25.1 ml H_2O.

10. Use a 10 ml syringe w/ needle needle to fill burette.

11. I have made a small hose funnel @ the top of the burette to make filling easier & safer.

12. You do not need larger syringes.

Good pH Titration Methods are now in place:
Let's repeat the test.
This time we have 5.4 ml acetic acid dissolved in 25.2 ml H_2O

$\frac{\text{count}}{\frac{1}{\text{ml}}} = \frac{\text{count}}{1}\,\text{ml}$

Initial pH is 1.76

		Vol
n = 41	pH = 3.27	~ 12.2 ml
n = 41	pH = 4.17	~ 12.1 ml
n = 41	pH = 8.07	~ 12.0 ml

Fastest pH change is between n = 31 pH 6.77
n = 32 pH 6.97 Δ = 20
$\bar{X}$ = : n = 31.5 pH = 6.87 equivalence point.

Volume $\bar{X} = (41 + 41 + 31.5)\left(\frac{36.3\text{ ml}}{123\text{ count}}\right) = \left(\frac{113.5}{\text{count}}\right)\left(\frac{.295\text{ ml}}{\text{count}}\right) = 33.5\text{ ml}$

Ok, here we have 33.5 ml of commercial ammonia is required to neutralize 5.4 ml of vinegar dissolved in 25.2 ml of H_2O.

And our equivalence point is estimated @ pH ≈ 6.87

Our previous equivalence point estimated @ pH = 6.14

$\bar{X} = 6.50$ Considering the pH is changing @ a rate of ~.20 per count

We have $\frac{\Delta pH}{\Delta \text{count}} = \frac{0.20 \text{ per count}}{0.295 \text{ ml per count}} \approx 0.68 \frac{\text{count}^2}{\text{ml}}$ Uncertainty

Our equivalence point is determined within ~ $\frac{0.6\text{ml}}{33.5\text{ml}} = 1.8\%$ Error in Volume Estimate

This is quite good, especially in fast mode.

I am setting up now the means to conduct the following procedures in a portable setting:

1. Liquid Chromatography

2. Titration (Power required (Amount?)
   (Low power mode is available also)
3. Near Infrared Spectrometry (reflectance)

4. VIS spectrometry

5. Electrochemistry

This is a fairly powerful combination.

The main methods missing are MID-IR & UV.

The semi automatic titrator eliminates a great deal of the tedium that can accompany that method.

Jan 5 2019

I want to look @ three items today:

1. Can I reduce the foot-print of the instrument.
2. Can I use my ORP probe?
3. Let's attempt a calibration & preparation of pH buffers.

---

1. OK, I have already improved and reduced the footprint (and lowered it) of the instrument. It looks much cleaner now. I also do not think that I need the funnel in addition to the pipette modification, so that reduces the foot print further. It looks cleaner. I also simplified the burette dispensary arrangement.

Trial again: Fast Mode - Make sure that you flush & prime the burette w/ titrant!

Initial pH = 1.92 ~Volume

n = 24 pH = 3.59 11.9ml

n = 24 pH = 4.63 12.0ml

Slow! n = 86 6.32 5.0 ml You do not want to be starting a new segment right @ the equivalence

n = 84 6.12 5.0 ml points.

Greatest change @ n = 5 pH = 6.45

n = 6 pH = 6.53

Equivalency pt = (6.45 + 6.53)/2 = 6.49 vs 6.50 avg - Superb.

Volume: ~~Fast:~~ Fast: ~~48/23.9~~ 23.9ml/48 counts = 0.498 ml/count

Slow: $\frac{10\,ml}{170\,counts} = .059\,\frac{ml}{count}$ ⇒ 23.9ml + 5.5(.059ml) ⇒ 24.225ml + 5ml

This is volume ⇒ (= 29.225ml)

Note that our new capillary seems to have a larger aperture since the FAST mode is delivering a light volume,

However, also notice that the slow speed remains identical to before. So therefore actually have a very good combination and the new capillary tube is just fine and the moment is for superior.

—

It also seems that we can use our ORP electrode just fine, It is not clear yet what the difference structurally is between the pH and the ORP meter since they both seem to be able to measure in millivolts.

Either way it seems we now have a total of 6 electrodes that can be used w/ the new titrator and that means that there is a lot of chemistry to explore here.

—

pH meters and ORP meter electrodes are indeed very similar, but not exactly the same thing. They are both measuring the tendency of a solution to gain or lose electrons. The main difference is the chemical inside the electrode that is used to create the voltaic cell w/in the solution.

We know pH is measuring H^+ ions.
ORP probably measures all ions.

Oxidizers increase the ORP (mostly disinfectants)
Reducers reduce the ORP. (mostly contaminants)

You are right, pH electrode measures only H^+.
ORP cannot discriminate between ion species

In general:
Increasing pH $\longleftrightarrow$ ORP decrease
from one paper
He did not say the converse.

So I say they really ARE NOT the same thing
And you need to ask yourself what it is that
you want to measure?

ORP → measure redox
pH → measure acid/base, ie H^+ ions

A single drop of bleach in water radically
increases the ORP. (rose to ~ 600 mV)

A drop of vinegar added to the bleach made it
even higher ~ 1050.

The says the combination of vinegar and bleach
is an incredibly powerful oxidizer.

Ammonia (a relatively large amount) drops
the ORP to about +850, or about midway
between the bleach and vinegar.

Thus gives us a better handle on the use of the pH/ORP electrodes.

Theoretically we want to combine our meters & collect measurements

pH
ORP
Conductivity

all at the same time and conduct a titration w.r.t. either pH or ORP

Ok, now we want to calibrate the titrator pH meter.
Certainly we will need buffers.

To calibrate the titrator we need a minimum of

1. One buffer w/ a pH of 6.86

Optionally we may have a second buffer of pH = 4.00

OR

a buffer of pH 9.18

Let us see if we can make something of 6.86.

1. Monosodium Phosphate MW = 119.98 gm/mol FW 137.99
NaH_2PO_4

The formula mass (formula weight) is the sum of the atomic weights in the empirical formula.

The molecular mass (molecular weight) of a molecule is the AVERAGE MASS as calculated by adding together the atomic weights of the atoms in the molecular formula.

Why is Ward's using formula weight?

2. Na_2HPO_4 ⇒ MW = 141.96 gms/mol
Sodium Phosphate Dibasic

Recipe for pH = 6.9 0.1 M Solution

= 14.302 gms Na_2HPO_4 (dibasic)
= 6.436 gms NaH_2PO_4 (monobasic)
in 200 ml H_2O, add H_2O to 1 liter
800

Buffer pH 9.2 0.1 M Solution
= Sodium Bicarbonate 7.644 gms
= Sodium Carbonate 0.954 gms
in 800 ml H_2O, add to 1 liter.

There is where the large syringes can be helpful.

Tuesday AM we do not need a liter of each buffer for now. 200 ml will be fine.

We bring the final volume to the reference.

So we only need:

pH 6.9: Mass of Jar = 184.72 gms

(0.2)(14.302) = 2.069 gms Dibasic
(0.2)(6.136) = 1.287 gms Monobasic
in 150 ml H_2O, bring to 200 ml

$\Sigma = 3.341$ ⇒ 200 ml − 3.347 gms = 196.65 gms H_2O

pH 9.2: Mass of Jar = 190.02 gms

(.2)(7.644) = 1.529 gms Bicarbonate
(.2)(.954) = .191 gms Carbonate
$\Sigma = 1.720$ gms

200 ml − 1.720 gms = 198.28 ml H_2O

I have completed the first pH Calibration of the instrument. I used the two point method in an attempt to get the best results. The phosphate buffer prepared @ a pH of 6.98 measured spot on @ ~6.96 pH.

The Carbonate buffer did read @ 8.3 instead of 9.2 @ the end of the second point, however the 6.98 buffer is displayed Calibrated.

One point Calibration @ 6.98 can also be made.

—

We are now in a position whereby once again we should be able to determine the identity of an unknown acid.

The first step would be to titrate vinegar (the analyte w/ a KOH solution (titrate) to determine the strength of the vinegar.

Once we know this, we could use the vinegar as the titrant to determine the strength of the ammonia.

Then we could assume we no longer know the identity of the vinegar and use the ammonia as titrant to hopefully determine the identity of the analyte (vinegar).

1M KOH solution = 56.11 gms / liter
.1M = 5.61 gms / liter

.1M KOH 200ml = $\frac{1.12 \text{ gms}}{200 \text{ ml } H_2O}$

*

Tube 3-* series

June 06 2019 - Liquid Chromatography Summary

I have improved the stability and centering of the fibata even further today - it is a clean looking unit now. There are two locations where the support rod can be mounted; one is certainly more stable than the other.

Let's summarize the most recent LC runs:

Tube 3-1

Protein negative (per concentration)
Ninhydrin Test (Amine) positive (per concentration)
Proposal: Aromatic polar amine compound
NIR: RNH_2, CH_2 or ROH proposed
IR plot available
SDBS consulted

Tube 3-2

Protein negative (per concentration)
Ninhydrin (amine) Test is positive (per concentration
NIR: RNH_2, CH_2 or CH_3 proposed
No IR plot apparent

Tube 3-3 Protein negative (per concentration) ~~(weak reaction)~~
Amine Test positive (weak reaction)
NIR RNH_2, CH_2 or ROH
IR Plot available, it does differ from Tube 3-1
Issue of pyrimidines addressed here

X

LC Summary - Tube 3-x Series

Tube 3-6

Protein test is positive.
Amine Test Negative (but conflicting results)
NIR: RNH_2 & ArCH proposed
IR Plot available

Tube 3-8

Protein test negative (per concentration)
Amine test negative (per concentration)
NIR - ArCH proposed
SOBS Consulted (per significant study)
Polymeric OH suggestion
IR plot available, preliminary analysis made
Naptha amine topic raised
Aromatic raised
Pyridines raised - DNA issues
Significant note here

Tube 3-12

OKV
It raises a lot of questions. Col Tube 3-12 assessment @ the close is that we definitely have a valid signal, even after subtracting the background reference solvent of ammonia.

We have a strong valid IR plot to interpret here.

No useful NIR data results from 3-12.
Protein test is negative. Ninhydrin test does show a shift in color from the ammonia control.

*

LC Summary - Tube 3-* Series

Tube 3-11 (& 3-10)
for 7 monumental importance.

Tube 3-11 is especially important. The required an especially difficult extraction because of the complex that forms in the column; very strong H_2SO_4 was required for the final 9 complete extractions

1. Protein test is Strongly positive

2. Ninhydrin (amine test is negative

3. NIR absorbance RNH_2, ArCH proposed

4. IR Plot available. Extensive notes have been made in this volume of the terms of analysis. The protein extraction matches the infamous "Environmental Filament" digestion that was recorded in Dec 2017.

5. There is a critical component to further analyze.

Let's look @ a redox titration.
Chrome Vit C + Iodine + starch

Well, that was quite interesting. The trial worked fairly best w/ a surprise.

We see that almost no Vit C is required, and a lot of 10% Povadone Iodine was required to effect a color change.

General method is:

1. Create a starch solution by boiling a small amount of starch in 25-30 ml of H_2O.

2. Add a very very small amount of Vit C (estimate .01-.02 gm is plenty) to about 25 ml of H_2O. Make about 200 ml @ this concentration, or about .05 gms / 200 ml
3. Lets make this the analyte. Add a little starch (3-4 drops) solution as a color indicator

3. You need a relatively concentrated iodine solution, I would estimate @ least 10 ml 10% Povadone in 50 ml H_2O or 20 ml Iodine in 100 ml H_2O.

Vit C Analyte
.05 Vit C
201.02 gms (ml) H_2O

Povadone Titrant
10.12 ml 10% Povadone
106.34 ml total volume H_2O

24.43 ml Vit C analyte.

Use ORP electrode - Fast Mode FAST = $\frac{11.9\,ml}{26} = \frac{450}{1}$

Initial mV = +148

		Volume
n = 8	~~mV = +156~~ +148 mV	
n = 8 + 18 = 26	+210 mV	~11.9 ml
n = 5	+416 mV	~2.6 ml

Ok, we see the color change took place between n = 3 & 4

n = 3 +200 mV $\bar{x} \cong$ 337 mV @ $n \cong 3.5$

n = 4 +394 mV

Therefore total volume $\cong$ 11.9 ml + 3.5 (~0.498 ml/count) $\cong$ 13.6 ml

There is sufficient information to determine the Conc of the titrant in the Cup, assuming 10% Provodore, the concentration of the analyte.

The process however, worked flawlessly, but I needed to switch to slow mode after the first burette (~12 ml) emptied. You also forgot to put in the magnetic stirring rod, initially.

Repeat. Vit C Analyte = 29.56 ~~gms~~ (ml)

Initial MV = +133 mV

		Volume	
n = 23	mV = +208 mV	~11.1 ml	FAST
n = 23	mV = +402 mV	~1.2 ml	SLOW

Δ max @ n = 19 (+289 mV) to n = 20 (+354 mV) $\bar{x}$ = +322 mV

@ Volume = 23 (~~.498 ml~~ .458) 11.1 ml + 19.5 (1.2 ml) = 12.9 ml

You forgot to initially added Sharon's OK-Sol it.

You show the information you need now to equate the two.

* Volume should be calculated, both fast & slow before any runs

Fast Calibration:

$\frac{12\,ml}{24\,counts} = \frac{x}{1}$ $\quad x = 0.50\,\frac{ml}{count}$ therefore

Flow Calibration:

$\frac{4\,ml}{72\,counts} = .056\,ml/count$

You can tighten the Hoffman clamp to slow down the rate of your work. Eg $\frac{12\,ml}{57\,counts} = 0.235\,\frac{ml}{count}$

Yes, that works well.

If you want to get optimum efficiency, you would set flow rate @ or $\frac{12\,ml}{90\,counts} = 0.133$

so about 0.2 ml/count would be a good setting

Set @, for example @: $\frac{12.0\,ml}{75\,counts} = \frac{0.16\,ml}{count}$

You have seen however, that the flow rate can vary w/ column pressure.

Test the idea.

Proceed up only $\frac{4}{3}$ ml instead of 12:

$\frac{4\text{ ml}}{17\text{ count}}$ = 0.235 ml vs 0.16 ml for 12 ml

The shows you that use enough, the flow rate is NOT CONSTANT in a gravity fed column. Your best results will be obtained by reading the volume directly. Technically you can determine the flow rate if you will just record the volume for each run. Otherwise you will introduce a large error into your computation.

The titrator does the following:

1. Stirs the solution
2. Dispenses titrant, actually @ a variable rate
3. Records any point of maximum change
4. Allows for a flexible flow rate.

It does not actually determine the flow rate accurately

Record the final Volume for each run.

The error will be less if the flow rate is high, however.

About 0.3 – 0.4 ml $\frac{}{\text{count}}$ will be better.

June 07 2019

An advantage of titration with the ORP electrode is that you really do not need the color indicator. You rarely have such an optic anyway so lets make a run w/ out the starch.

Also the Near Infrared instrument has come in today. The small unit holds potential for many promising applications.

Although the instrument is made solely for diffuse reflectance work on solids I have already devised a plan and method to see if I can get a signal through a liquid. It is essentially an idea to make a microscope slide cuvette w/ a spacing of ~ 3 mm between 2 glass slides sealed on 3 edges w/ silicone. I have made four such custom cuvettes that I will attempt to use.

I have not actually opened up the instrument as we get. The legal and the project idea I obtained from a webinar set up by the vendor for the instrument.

The NIR portable instrument is working perfectly. Ceramic tile makes an excellent reference spectrum. As with Mid IR, the more water you can get out of the sample, the better.

Example, 10% Povidone Iodine gives no discernable signal in a path of ~ 3 mm. The cuvettes do not work well and they are not necessary. NIR also does better w water removed although it can also be used to quantify water content.

Evaporated Povidone gives an excellent sample. A thick evaporated sample gives a much better spectrum than a thin one.

We have NIR absorbance @:

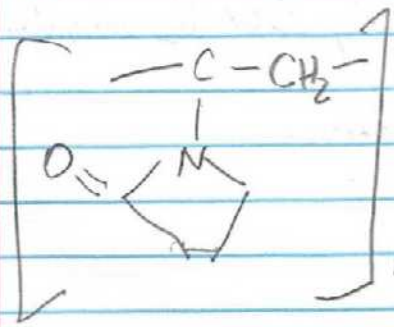
1191 nm moderate — CH

1439 nm (strong) — $CONH_2$ $CONHR$

1560 nm (weaker) — None (comb overtone presumed)

1700 nm (very strong) — CH_2

The actual structure is:

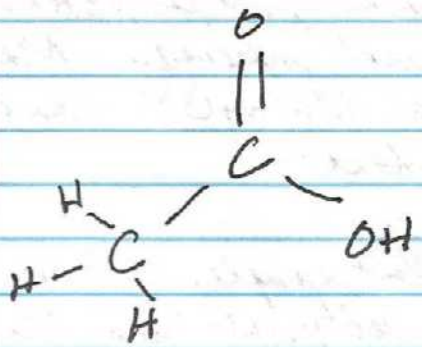


Notice how NIR worked supremely well here.

I also have worked up evaporated acetic acid (the signal and sample like are weak).

I pick up either CH or CH_2. Not real.

Here is the structure:



O=C can never be picked up by NIR, only hydrogen bonds.

Also C-O can not be picked up.

My only strong absorbance is @ ~1190, but this is not so superior to attempting w/ liquid.

The CDB Solvent Protein, by the way, gives the following NIR absorbance

970 nm (weak) (moderate) ArOH possible

1176 (moderate) CH_2, CH_3, CH

1460 (very strong) CONHR, $CONH_2$ possible

Aromatic, polar, protein, aliphatic characteristics are most likely.

We now have a solid NIR spectrum of the CDB Solid Culture Growth. (Mostly dehydrated, some moisture left, glass slide or ceramic.)

There is absorbance @:

1445 (very strong) $CONH_2$, ROH, CONHR

1180 (weak) CH_2, CH, CH_3

925 (moderate) CH, ROH

Protein, aliphatic chains, molecularly leads to unlikelihood of ROH

—

after the drying of the sample, NIR scans are incredibly simple & fast to complete.

—

NIR on the CDB culture solids dissolved in strong HCl

1180 strong CH_2, CH_3, CH, ~~ROH (not suitable)~~
1459 strong CONHR, $CONH_2$ ROH (not suitable non polar)

Essentially the same results as the evaporated CDB solids, it shows that HCl had no effect upon NIR absorption

June 08 2019

I have done some strategic thinking this morning on the state of affairs with respect to CarrCom Institute.

My thinking leads me to access the state of affairs to fall into three different categories.

1. Big Picture Issues
2. Intermediate issues
3. Day to day operations - Immediate Progress Issues

Incidentally, before proceeding, I notice this morning that the public is beginning to get wind of the patent application that was filed in Sep 2015 with the inventor name of Clifford E Carrcom.

The information in my current laboratory notebooks far outstrips the state of knowledge given at that time.

Refer to the Santa Fe Conference for some phebelic comments made regarding the status of the particular intellectual property.

Continuing, the three categories, in more detail, include the following:

1. Big Picture Issues:
   1. Create a new non-profit - or corporation (?)
      Carnicom Foundation
   2. Submit a IRS 1023 seeking federal approval
   3. DNA analysis - Potential road blocks ahead - one company has refused to process the samples thus far.
   4. Laboratory books indexed
      Publicly published Vol 2 - Vol 22
      Volumes ≥ 22 are to be released only to the future Carnicom Foundation - significant intellectual property within.
      or made available publicly posthumously
   5. Community Health Professional Network (~~CHPN~~) (CHPN) leadership established.

This is the Difficult Section:

2. Intermediate Issues

Tremendous Capability, expertise & skill are now in place at Carnicom Institute along w/ a fabulously resourceful & reasonably equipped laboratory. It has taken a great deal to get to this point; a few struggles we might admit.

Capabilities now include, but are not limited to:

1. Mid-infrared instrumentation, methods & library
2. Near Infrared (NIR) - brand new Capability here
3. UV spectroscopy
4. VIS spectroscopy
5. Advanced electrochemical technology including voltammetry, impedance spectroscopy, etc - esp. for inorganic analysis
6. Gas Chromatography
7. Liquid chromatography separation (Column)
8. Automatic Titration (recent addition)
9. Manual Titration - Conductivity, pH, ORP methods)
10. Osmometry - Molecular Weight Determination
11. Distillation Techniques
12. Advanced Microscopy (up to 8000x)
13. Culturing & Incubation Methods
14. Aquarium Biology Research
15. General Qualitative & Quantitative Chemistry Methods
16. Centrifugation, VACUUM & Microwave applications
17. Combustion & Fume Hood (Not fully enclosed)

Now that this fantastic accomplishment is in place,

To what best end is the facility & staff (i.e., me) suited for?

What exactly would we like to accomplish with it in the foreseeable future?

What will be the disposition of the facility and its resources (and the knowledge base that has accumulated from its existence after I pass?

What is the purpose that this laboratory will serve in the future?

The intermediate issues and questions are the most difficult of the bunch. This is namely because they are operating w/in an indefinite time frame, and are immediately affected by

1. My existence
2. Adequate funding to maintain stability, let alone advancement
3. Knowledge transfer from me to whom?
4. How is the work accomplished to be taken advantage of?
5. Who will take advantage of the work and how will I be certain that it is put to serve the best interests of the public?

I do not have the immediate answers

to the Intermediate Issues

but you can be certain that I seek them.

The answers to these questions serve
as the bridges between the
Big Picture Issues and the Day to Day
~~Progress~~ Operations & Immediate
Progress Issues.

1. Big Picture Issues are conceptually
clear. It is simple for me to
visualize & establish this plan.

2. Intermediate Issues are the greatest
ones to struggle with - these
deal w/ the realistic limitations in
the current environment and state
of society.

3. The Day to Day operations and Immediate
Progress issues are also, once again,
very easy to deal with.

It is in this category that the actual
patient & persistent progress is made,
and it is how the knowledge I have spoken
of has come to be.

3. Day to Day Operations & Immediate Progress Issues.

Although this category can also be overwhelming to a single person, I am able to manage the set of needs relatively well. It is a situation of continually evolving interests and project priorities. The is when the work is actually accomplished and whatever has been recorded is of potential benefit to all in the future.

The current listing (a typical set) involves the following active projects under continuous prioritization (these are not in any order):

1. Liquid Chromatography of the soluble protein (2nd session)
2. Can yw once again Extract lipids from the COB.
   Need to locate previous work of yours past involving enzyme use
3. Category sample received. Numerous and usually a grab bag that requires evaluation & sorting to discern wh show significant enough interest.
4. Mollusk (pond snail) Toxicity Study
5. Edit and prepare the Santa Fe Conference video online (A significant undertaking).
6. Index laboratory notebooks - an ongoing saga
   Out of Vols 1-22, 14-22 have been indexed and are online.
7. Titration method development, study, and application projects identified.

8. Near Infrared (NIR) method development, study, and application projects identified and established
9. DNA extraction again?
   Correspond w/ the requesting labs?
10. Lipid extraction method developed?
11. Osmometer brought into operation again?
12. Electrochemistry – method development, study and application projects identified and established.
13. The Brain Wave project is certainly an intriguing prospect.

And this, then, is the state of affairs w/ CI.

1. Day to day work will continue, chipping away on the list of 13+ above as I see fit.

2. The reality of the "struggle" on the Intermediate Level will continue – maintaining a holding an optimistic attitude will help your Quest daily.

3. The Big Picture – Two years are being allotted to see significant progress on that front.

Liquid (Column) Chromatography (LC)
Soluble Complex LC Series

The Day to Day Operation Continues...

LC Run on the Soluble Protein Complex "4" Series.

	t	EC	pH	ORP	Flow Detect	Solvent
Reference Point	1405	NA	9.60	+113	4/2 = 2	H_2O

The column slowed down dramatically from a water flushing when the COB soluble protein complex was added to the column. The complex can be seen descending the column gradually. Water has now been added to the column and it immediately began flowing freely.

Tube 4-1	1430	NA	7.6	-83	6/2 ≈ 3	H_2O

Notice we have a fairly strong acidic compound here that is acting as a reducing agent. Recall our earlier work on the identification of sulfuric acid via titration. It is interesting that we have a reducing agent here H_2SO_4 is stated to only be able to act as an oxidizing agent. Also note that the pH of the separation is near neutral.

Recommend usual Protein, amine, NIR (700–1100 nm) for liquid, IR testing. Minimal sample available. It also means that the separation is a shoe-in to set up a redox titration with.

Tube 4-2	1450	6.06	7.54	-130	6/2 ≈ 3	H_2O

Notice it continues as ~~an~~ a strong, weak reducing compound. Neutrality of pH holds relatively constant. Notice also high electrical conductivity is in place. Should remain strongly related to Tube 4-1.

We notice also now that we have some color separation in the columns. Appears to be somewhat of a blue-grey separation @ the bottom of the columns. Tan layer remains @ the top.

Tube 4-1 forms a very defensive yellow layer on the ATR plate. That means that it may also be suitable for NIR

—

Our closest match for Tube ~~3-1~~ 4-1 is the solid form of the culture that was dissolved in strong HCl and then was subsequently neutralized for use on the ATR plate.

$r = 0.94$ so it is highly similar. It does seem to show a stronger aspect of the thiocyanate group, however.

It also shows a shift to ~3214 cm^{-1} from 3350 cm^{-1} and also a stronger absorption peak @ ~1350 cm^{-1}.

This also suggests there can be further separation to take place due because of the significant results that took place from the separating the strong HCl dissolving of the solid protein complex.

It is recommended that we work w/ a smaller amount of material introduced into the next column run and slow down the separation process. We may have eluted too much material too quickly on the first separation of the soluble protein.

Interesting, but it looks like a separation has taken place even w/in the column reservoir. Clear liquid is immediately above the column substrate top, however a tan or rust color dominates the majority of the fluid in the reservoir. The colored layer (presumably due to the oxidation of iron) is floating on top. This suggests that it is a more non-polar compound separation that now exists within the reservoir. Our indicators (electrochemical) show nothing of drastic change taking place.

It looks as though there remains plenty of material left to attempt separations upon.

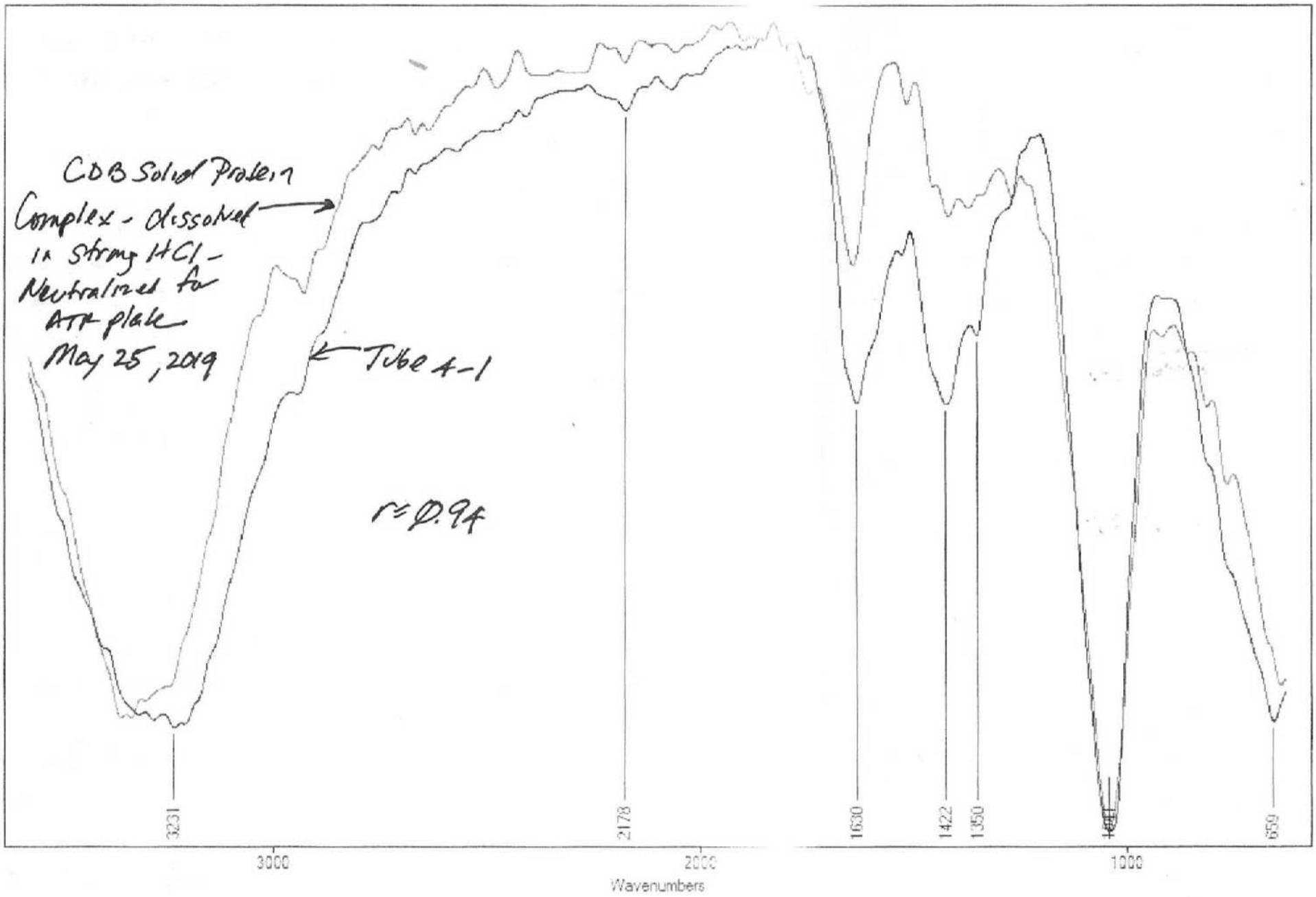
1. Less polar layer possible
2. Precipitation layer @ top of column possible
3. Blue gray layer @ bottom of column possible
4. Current layer in play here (may remain the same as Tube 4-1 & 4-2.

Tube 4-1 IR Comparison to Solid Protein Complex

CDB Soluble Protein - LC Separation - Tube

printed on 06/08/2019 16:26:00

CDB Soluble Protein - LC Separation - Tube 4-1 - Jun 08 2019 - 01.spc: Synthetic spectrum by averaging. See audit trail for details



printed on 06/08/2019 16:26:00

	t	EC	pH	ORP	Flow	Solvent
Tube 4-3	1605	1.55	7.23	-128	2/3 = 0.66	H_2O

Notice the separation remains a reducing Compound. pH remains near neutral. I suspect separation remains akin to Tubes 4-1 & 4-2.

We see from IR that Tubes 4-1 & 4-2 are essentially the same in all respects and that film layer formation on the ATR plates are the same. We shall therefore combine the two tubes into 4-1.

Noticed increased precipitation is gradually developing @ the top of the Column. It appear identical to the last layer of separation that was extracted only w/ the use of strong H_2SO_4.

Tube 4-3 is the same (by IR) as the now combined Tube 4-1, except that it is likely to be weaker in Concentration.

The Column is therefore stabilizing w/ the use of H_2O as the solvent, and it is time to switch to a slightly less polar solvent, isopropanol. Let's empty the Column from the remaining H_2O.

With the addition of isopropanol, the Column Continues to intensify in its existing Colors.

I am getting much better @ the separation process and also recognizing the points of change along w/ the transitions that are taking place.

We are now shifting into a positive ORP mode.

	t	Ec	pH	ORP	Flow	Solvent
~~Tube 4-4~~						
Tube 4-4	2135	.01	7.48	+114	36 18½/1=36	Isopropanol 70%

The blue-gray layer has now turned to dark tan after the addition of isopropanol

There is no sign visually or electrochemically that anything has come out in isopropanol.

Indeed nothing whatsoever shows up on the ATR plate after evaporation. From odor and the result we must presume the elution is almost entirely isopropanol. Flush the column empty.

Methanol is actually more polar than isopropanol so I see no reason to take on that route. But let's try for kicks.

	t	Ec	pH	ORP	Flow	Solvent
Tube 4-5						Methanol

Same result – no sign of any compound being eluted on the ATR plate. Ruled to vinegar.

The compound is highly polar as is. No advantage then to try eluting at a less polar composition.

	t	EC	pH	ORP	Flow	Solvent
Tube 4-6	2230	~2.1	6.19	+125	~5	Acetic Acid

As expected, acid disrupts the column to some degree; some flow is always required to release gas buildup.

Tube 4-6 still has a strong alcohol odor so it is a mix between alcohol & acetic acid. The column is becoming lighter in color as vinegar passes through so we know that some level of separation is taking place.

Tube 4-6 evaporates to a crystalline nature on the ATR plate. Much stronger than the evaporation of vinegar alone. So we know that we have a compound of some form. Also because of mix of vinegar w/ residual alcohol in the column, the sample on the ATR plate evaporates more quickly.

I believe Tube 4-6 is likely to be primarily an inorganic compound. IR plot available on next page.

	t	EC	pH	ORP	Flow	Solvent
Tube 4-1	2320		5.14	+203	6/2 = 3	Vinegar

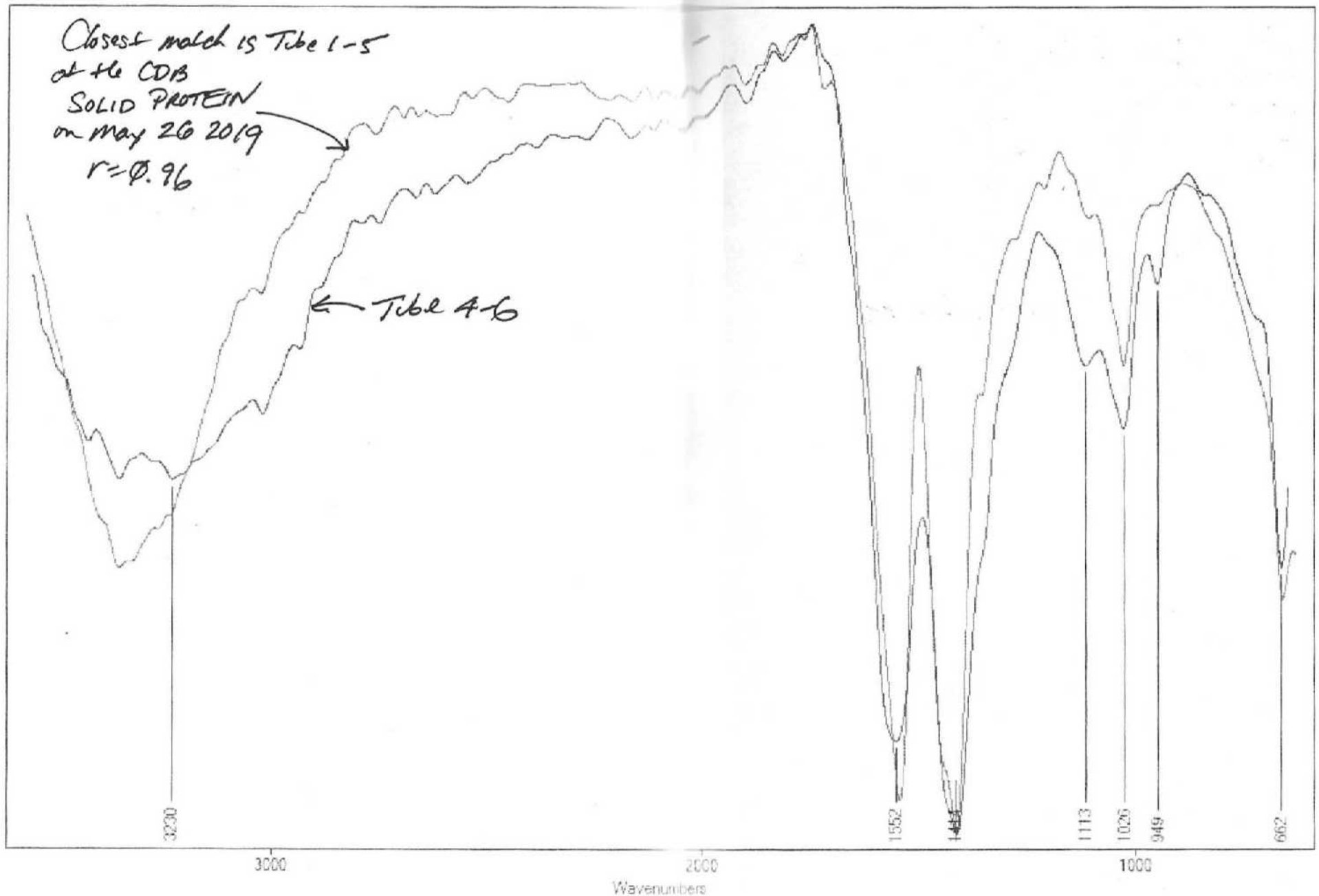
Very slow column. No obvious elution. Small sample.

As we say, be VERY CAREFUL about assumptions. Even though Tube 4-1 elution is perfectly transparent, we most likely have a compound.

Tube 4-6

CDB Solu

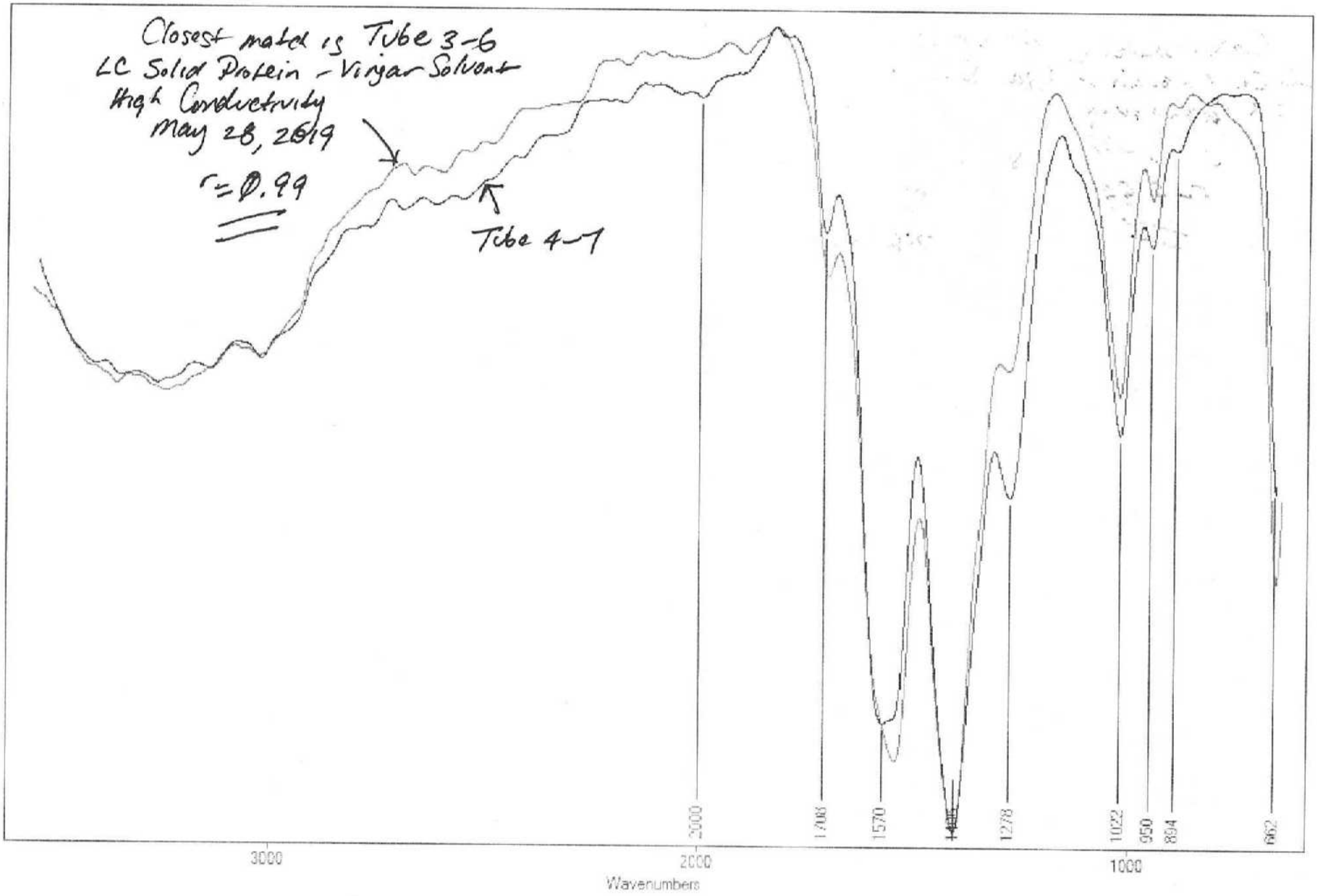
printed or


Closest match is Tube 1-5
at the CDB
SOLID PROTEIN
on May 26 2019
r=0.96
← Tube 4-6
3230
1552
1113
1026
949
662
3000
2000
1000
Wavenumbers

Tube 4-1

CDB Soluble Prot

printed on 06/09/


Closest match is Tube 3-6
LC Solid Protein - Virgin Solvent
High Conductivity
May 28, 2019
r = 0.99
Tube 4-7
2000
1408
1570
1278
1022
950
894
662
3000
2000
1000
Wavenumbers

The separation seems to be especially pure. At evaporate to a completely transparent crystal on the ATR plate. Notice that Tube 4-7 does have a couple of difference from Tube 4-6.

Notice also that Tube 4-7 has also essentially a perfect match with Tube 3-6 FROM THE SOLID PROTEIN.

We are seeing crossovers and common ground between the soluble and insoluble CDB growth complexes. This has an element of surprise to me.

We continue w/ vinegar until we know that the Column is stable w/ the eluate.

Our strongest peak w/ Tube 4-1 is 1406 cm^{-1}.

Parker has these as
1440 – 1350 strong S=O $(RO_2)SO_2$

sulfuric ester

1420 – 1330 strong S=O $ROSO_2E'$

sulfonic ester

Does not look like any real competition here.

ala @ ~ 1560 - 1540

~1585 amine salt (medium)
1540-1515 strong NH Amide II (solid)
1550-1510 strong NH secondary amide (dilute solution)
1580-1520 med. C=N, plus C=C pyrimidines

Notice band location is closest to pyrimidines candidate

Pyrimidines w/ sulfur a sulfonic ester is a topic here. Amide & amine tests will be important.

The protein reagent is:

200 ml Ritz Red Dye in 12 ml H_2O (stock solution)

so: PROTEIN REAGENT:

1. 3 ml H_2O
2. 1 drop 10M NaOH
3. 2 drops 0.5M $CuSO_4$
4. 100 ml stock solution red dye
5. Pinch Cream of Tartar

Ok, a big surprise to me. Tube 3-T passed the protein test immediately w/ flying colors. The dye only gives w the amide. However, notice the absorption band is unusually wide, and extends most certainly beyond the amide IN SOLUTION. I'd therefore place pyrimidines also as an equal priority.

4-7

The analysis indicate that in Tube ~~3~~ 4:

1. We almost certainly have a protein
(Colorimetric test and IR support this)

Still questions here → 2. Pyrimidine in Conjunction w/ amide absorption ??

3. Sulfonic or Sulfuric ester

As only a preliminary assessment.
Please review notes on Tube 3-6 previous
review per notes on IR Comparison.

The looks to be a very unique, completely
transparent & clear compound.

The amine test is absolutely negative. We
know we have the amide.

Test for an ester would be wise here again.

A Test for esters was included in the notes
of May 21 2019.

Ok, very interesting results for the ester test,
even without employing the hot water bath.

If you review the test method

Pink should stay	Pink should decrease
Control Tube	Ester Tube
1. KOH (2 drops 10M KOH)	1. KOH (Used 2 drops 10M KOH)
2. Phenolphtalein	2. Phenolphtalein
3. Hot Water Bath - Boiling	3. Hot water bath - Boiling
~1½ ml H_2O	4. Suspected Ester
	~1½ ml H_2O

I never even made it to the hot wate bath. The tube w/ the ester never even made it to pink. It was already acidic. However, remember Vinegar was being used as the solvent. But also understand I am using 10M KOH.

It has required 4 drops of (10M KOH) to neutralize the acid and turn the indicator pink. The seems like a lot of very strong acid. But now lets go ahead w/ the hot water bath and also try a phenolphtalein test w/ vinegar alone as a control.

It required 14 drops of 10M KOH to turn a ½ ml vial of vinegar pink w/ 1 drop of phenolphtalein added. The tells us that the acetic acid concentration in the ester test vial was very low, We may therefore have a combination of effects taky place there. Nevertheless, let do continue w/ the hot water bath test.

"Esters have characteristic smells and are insoluble in water"

(We are in acetic acid (?).

Compounds in which the -OH of the carboxyl group is replaced by certain other groups are called carboxylic acid derivatives, the most important of which include the esters and the amides.

$R-\underset{\underset{O}{\|}}{C}-Z$

Z = OH = Carboxylic acid

$Z = NH_2, NHR', NR'_2$ = amides

* Acetic acid (vinegar) is a carboxylic acid.

Z = OR' = ester

Usually esters are derived from a carboxylic acid and an alcohol (notice that we were using acetic acid + methanol!)

They may well have formed a sulfuric or sulfonic ester, therefore?

Indeed the Ester Candidate vial is lighter pink just as the test outlines.

I therefore conclude that we do have an ester (sulfuric or sulfonic) that has formed, in conjunction with the protein of Tube 4-T.

There is, in the literature, reference to "Ester transfer proteins".

So proteins can be linked w/ esters.

We notice also that the protein precipitate in the vial because it has been subjected to alkaline conditions.

There may well be a method to isolate/separate the protein from the ester.

	t	Ec	pH	ORP	Flow	Solvent
Tube 4-B	0200	12.38	5.26	+101	3/2=1.5	Vinegar

Once again, Tube 4-B leaves a perfectly clear film on the ATR plate. All signs are that Tube 4-B is essentially equivalent to Tube 4-T. IR plot follows.

Notice we have a single strong peak near 1539. Well within the amide absorption band.

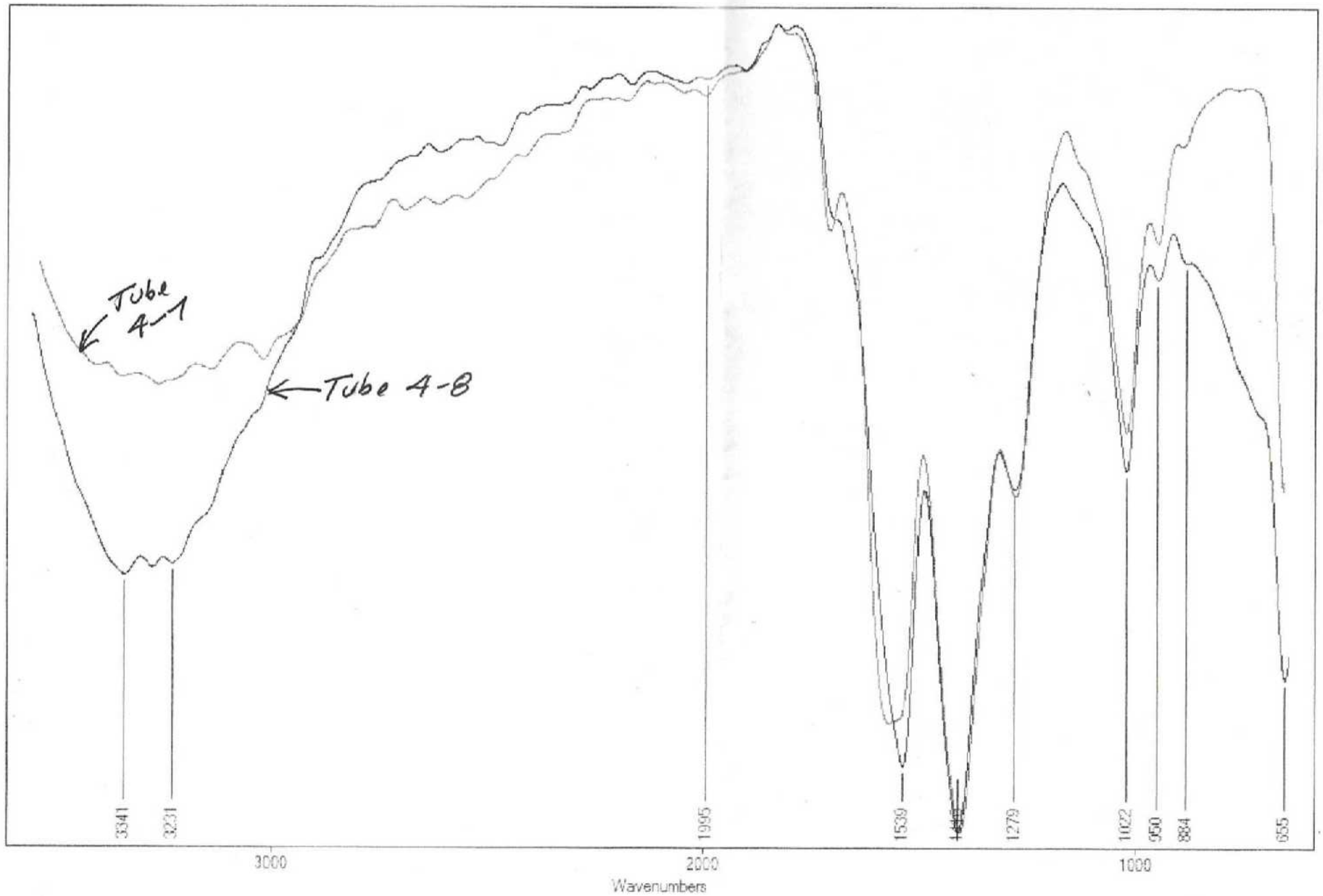
Continue w/ vinegar until the disappears

Tube A-B

CDB Soluble Protein -

printed on 06/09/2019

CDB Soluble Protein - LC Separation - Tube 4-8 - Jun 08 2019 - 05.spc: 06/09/2019 03:19:13 title



printed on 06/09/2019 03:21:27

June 09 2019 - June 10, 2019

	t	EC	pH	ORP	Flow	Solvent
Tube 4-9	2350	15.89	5.43	+164		Vinegar
	To be IR analyzed					
Tube 4-10	0200	11.20	7.71	+139	40/3=13	Ammonia
	To be IR analyzed					
Tube 4-11	0210	15.60	13.29	-125	24/3=8	Ammonia
	To be IR analyzed. Notice Reducer status.					
Tube 4-12	0920	17.53	13.57	-115	10/3=3.3	Ammonia
	To be IR analyzed.					

Observation: Tube 4-10 has a significant amount of pure white precipitate that has formed settled @ the bottom of the tube overnight.

We also recall by observation that tube 3-18 from previous run of the SOLID Complex also has a significant white precipitate that has settled @ the bottom of the tube that appears similar / identical. Tube 3-18 has a slight yellowish cast due to it.

Tube 3-17 & 3-18 are in the strong protein category. We note that Tube 3-17 (already tested positive for protein) is transparent & clear. Tube 3-18 has a yellowish cast to solution but also transparent & clear, but with white precipitate settled @ bottom of tube.

Notice Tube 4-10 was the switchover from vinegar to ammonia (pH 5.43 → 7.74)

—

Tube 4-9 is identical to Tube 4-8, Tube 4-8 is identical to Tube 4-7.

We have a significant amount of protein and concentrated chemical matter now within Tubes 4-7 to 4-9.

We have the identical clear transparent film that forms on the ATR plate during evaporation for IR runs.

Tube 4-7 thru 4-9 are each based upon the use of acetic acid (vinegar) as the eluent.

—

IR: Tube 4-10 will begin the ammonia run.

—

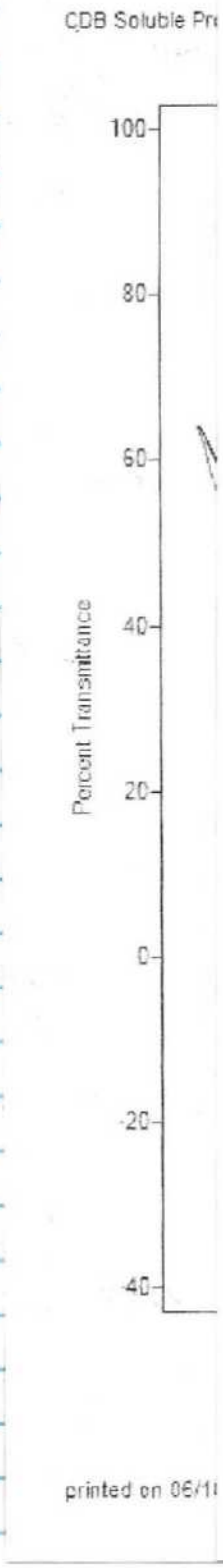
	t	Ea	pH	ORP	Flow	Solvent
Tube 4-13	1035	18.60	13.60	-119	8/3=2.7	Ammonia

We see here that the column has stabilized.

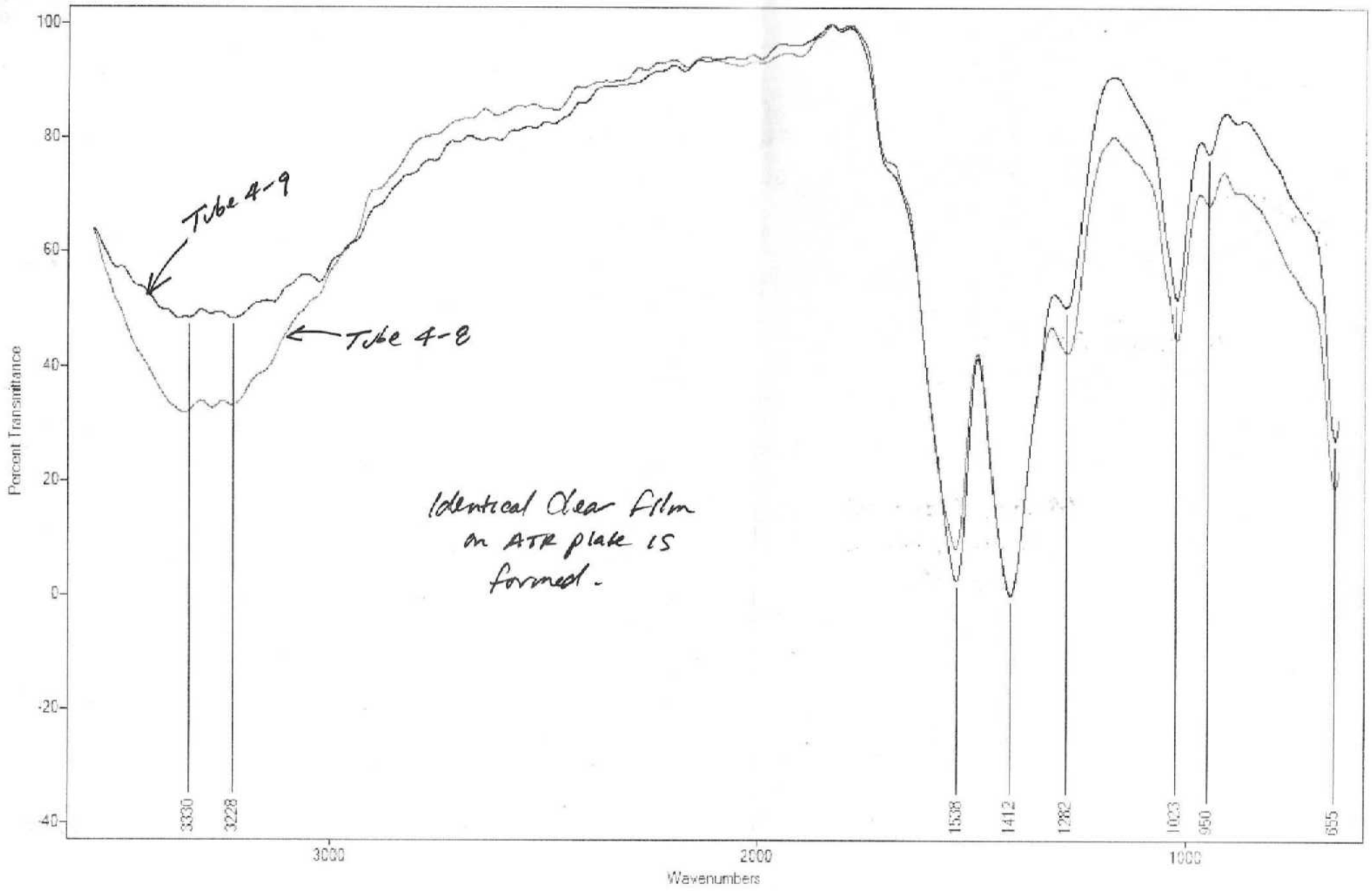
Tube 4-14	1130	18.58	13.63	-121		Ammonia

The columns are stable. Eliminate 4-13 & 4-14 from fourth analysis. Must now switch to H_2SO_4 to remove residual complex.

Tube 4-9 & Comparison to Tube 4-B.



CDB Soluble Protein - LC Separation - Tube 4-9 - Jun 10 2019 - 06.spc: 06/10/2019 11:17:16 title



printed on 06/10/2019 11:20:11

Protein Reagent: Modifications

	t	EC	pH	ORP	Solvent
Tube 4-15	120	~12	~6.5	~+500	H_2SO_4

Immediate cleaning of the column has taken place w/ the use of H_2SO_4. Extremely effective in removing residue from the column.

Let's conduct a protein colorometric test on Tube 4-15. Tube 4-15 is an entirely different compound than Tube 4-9. (Tubes 13 & 14 are removed from the extended analysis).

Protein Reagent: 10-12 ml H_2O is OK
Stock Dye: ~~200 ul~~ in 12 ml H_2O
300 ul also looks to be quite fine if not preferred. - red dye

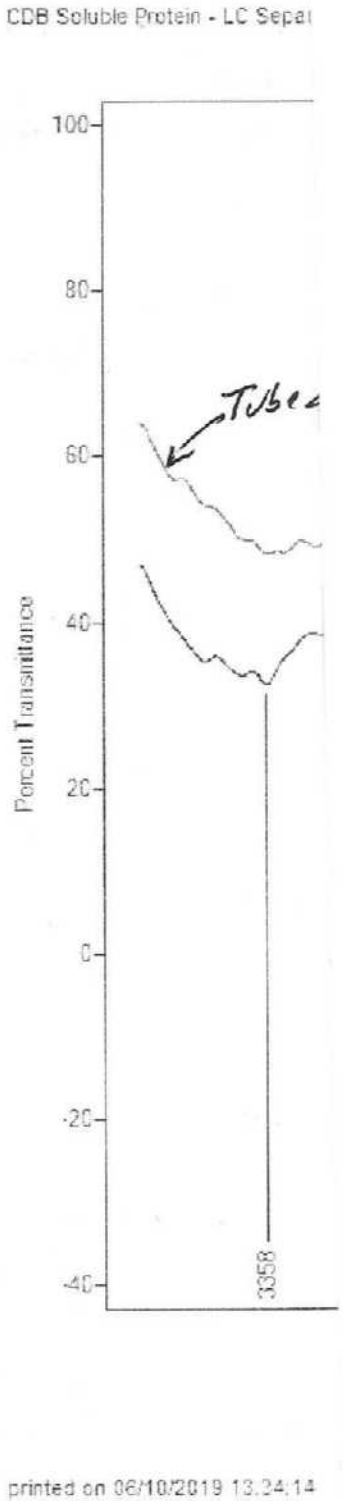
Then: 2 ml is sufficient
1. 2 ml H_2O & 100 ul Stock Red Dye
2. 1 drop 10M NaOH
3. 2 drops 0.5 M $CuSO_4$
4. Pinch Cream of tartar

Void
Revised

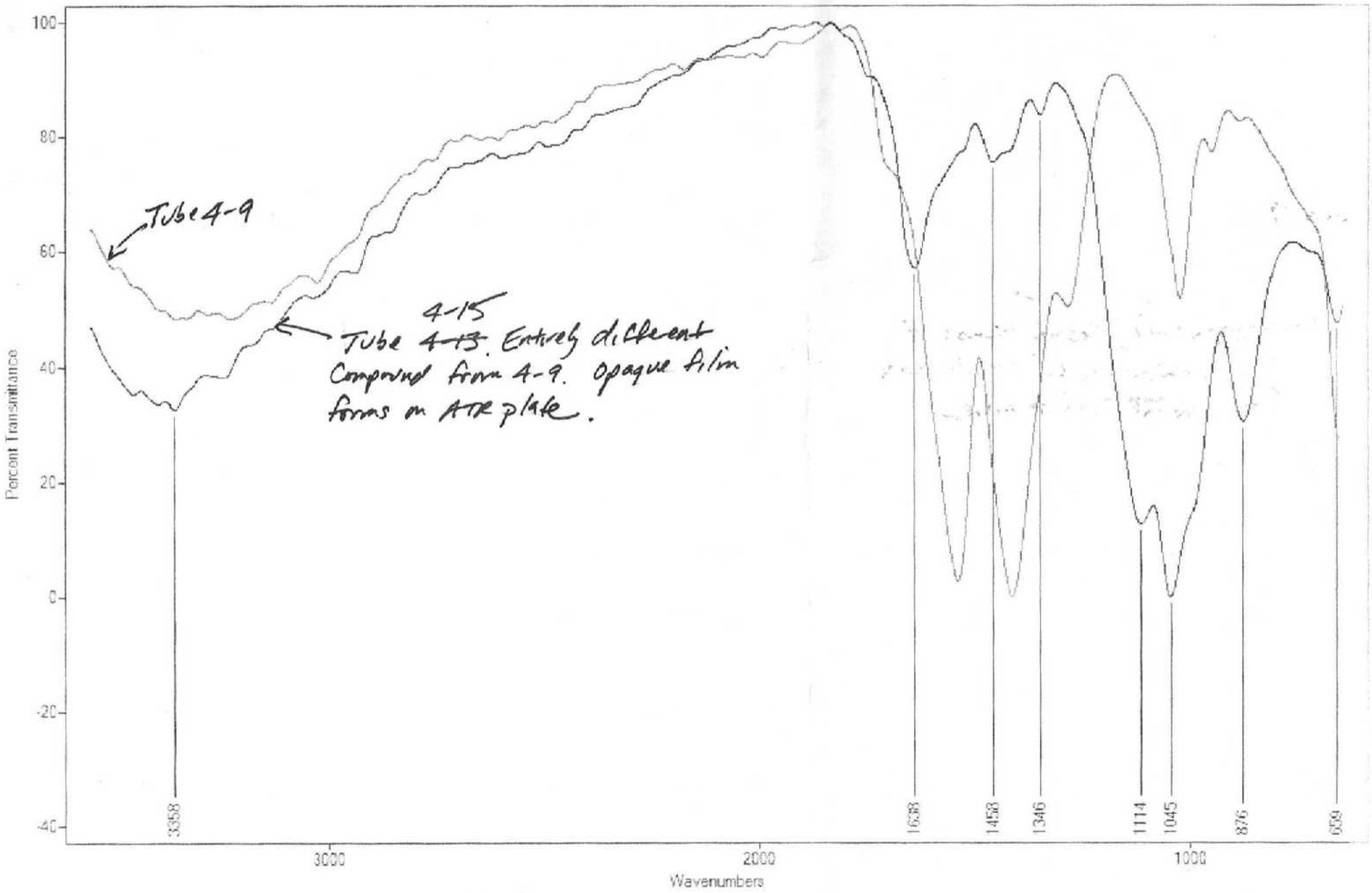
Tube 4-15 does not pass the colorometric protein test @ the concentration indicated.

If the dye is too thick to use the pipette you may have to add 1 drop of H_2O to the raw red dye.

Tube 4-15, Comparison to Tube 4-9. Completely different.



CDB Soluble Protein - LC Separation - Tube ~~4-13~~ 4-15 - Jun 10 2019 - 10.spc: 06/10/2019 13:31:35 title



printed on 06/10/2019 13:24:14

I have examined Tube 4-15 more closely with regard to the protein colorimetric test.

First of, Tube 4-15 has a mix of both solution and some precipitate. The solution retains some turbidity even after settling as well as centrifugation.

There is a slight shift in color towards purple but it is difficult to separate from the effects of turbidity.

The approach to the problem has been to centrifuge both the control sample & the Tube 4-15 sample. This leads to a majority of the dye compound settling portion to the bottom of the tube on both accounts. Here it can be seen a definite shift in the color of the centrifuged dye towards purple in the Tube 4-15 sample.

At this point, the protein colorimetric test is presumed to be positive for Tube 4-15, i.e. a positive indication for protein.

The amine test is negative, although we do observe a precipitation taking place w/en the heated tube w/ ninhydrin.

The interpretation of the observation is that we are most likely dealing with the denaturation of a protein complex due to the subjection by heat.

Our next tasks are:

1. Complete IR analysis on unexamined tubes
2. Run through the series systematically on colorimetric protein tests
3. Run through the series for amine presence
4. Conduct NIR analysis (two types)
   1. 700-1100nm - solution analysis w/UV-VIS-NIR spectrometer
   2. Attempt film analysis NIR manual diffuse reflectance 900nm-1700nm.
5. IR analysis as interest and time warrant
6. SDBS IR analysis as time and interest allow.
7. Engage in redox titrations for various samples - both as a learning exercise as well as application and interpretation development.

June 11, 2019

The film documentary crew is here today.

They are working on a documentary to capture some of the science here.

Some analysis has taken place today under the microscope of some unusual biological samples:

1. Filterous network that has developed from the toenail. Under the scope @ 320x it satisfies all requirements of the "Morgellons" condition.

2. ~~Insect~~ Insect specimens with the CDB filamentous interwoven amongst them. As a mensuum, insects are serving as a transport mechanism. Quite possibly more.

3. A hexagonal crystal, gold blue, has been observed amongst one of the insect sets. I have seen one of these crystals many years ago and they are reported by individuals frequently on the internet discussion boards.

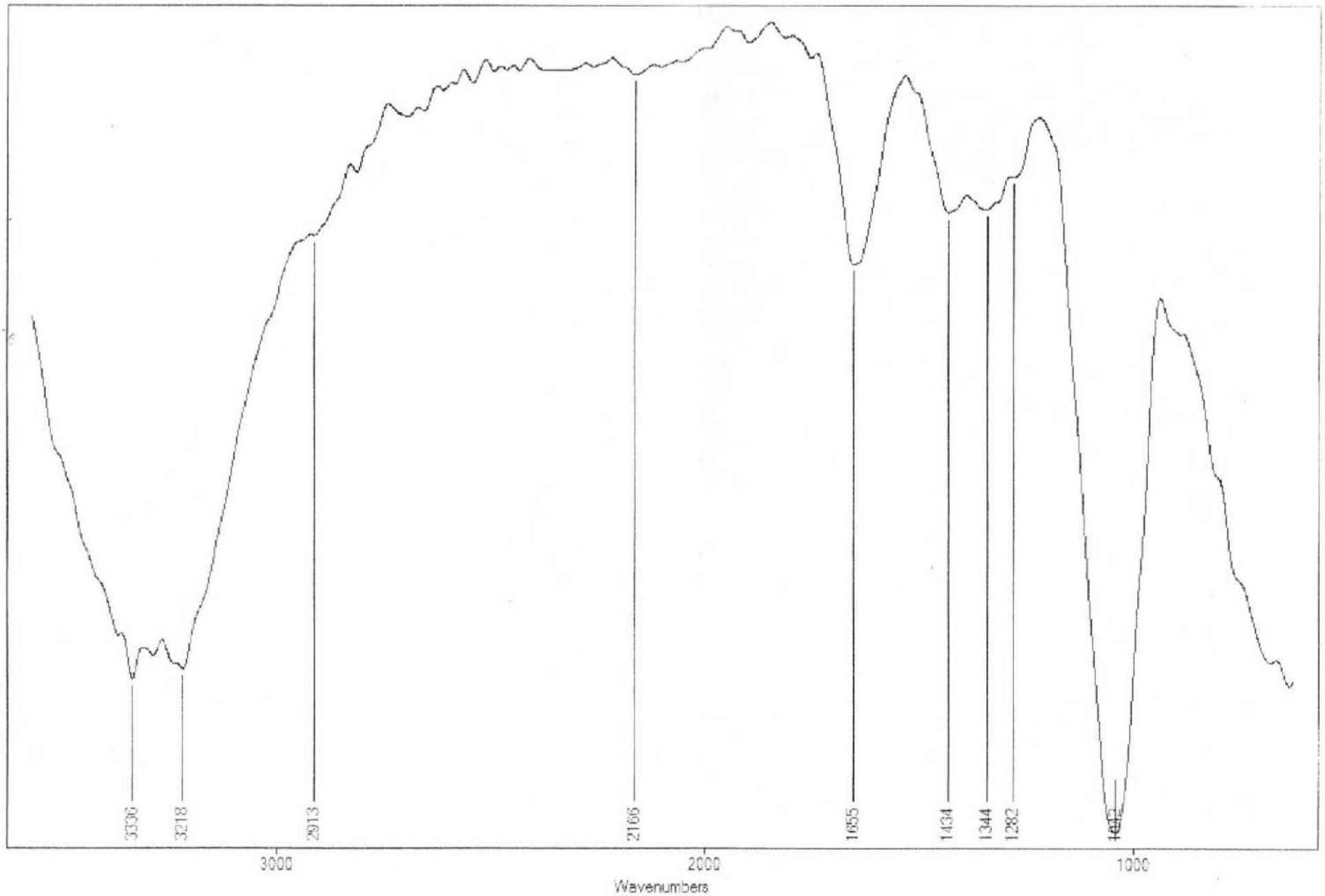
I am running a small sample of the soluble protein complex. The column needs to be run slowly.

reference		8.02	+258		
	EC	pH	ORP	solvent	Flow
Tube 5-1	6.2145	6.31	5.67	+186	H_2O	$32/3 \approx 10$

CDB Soluble P

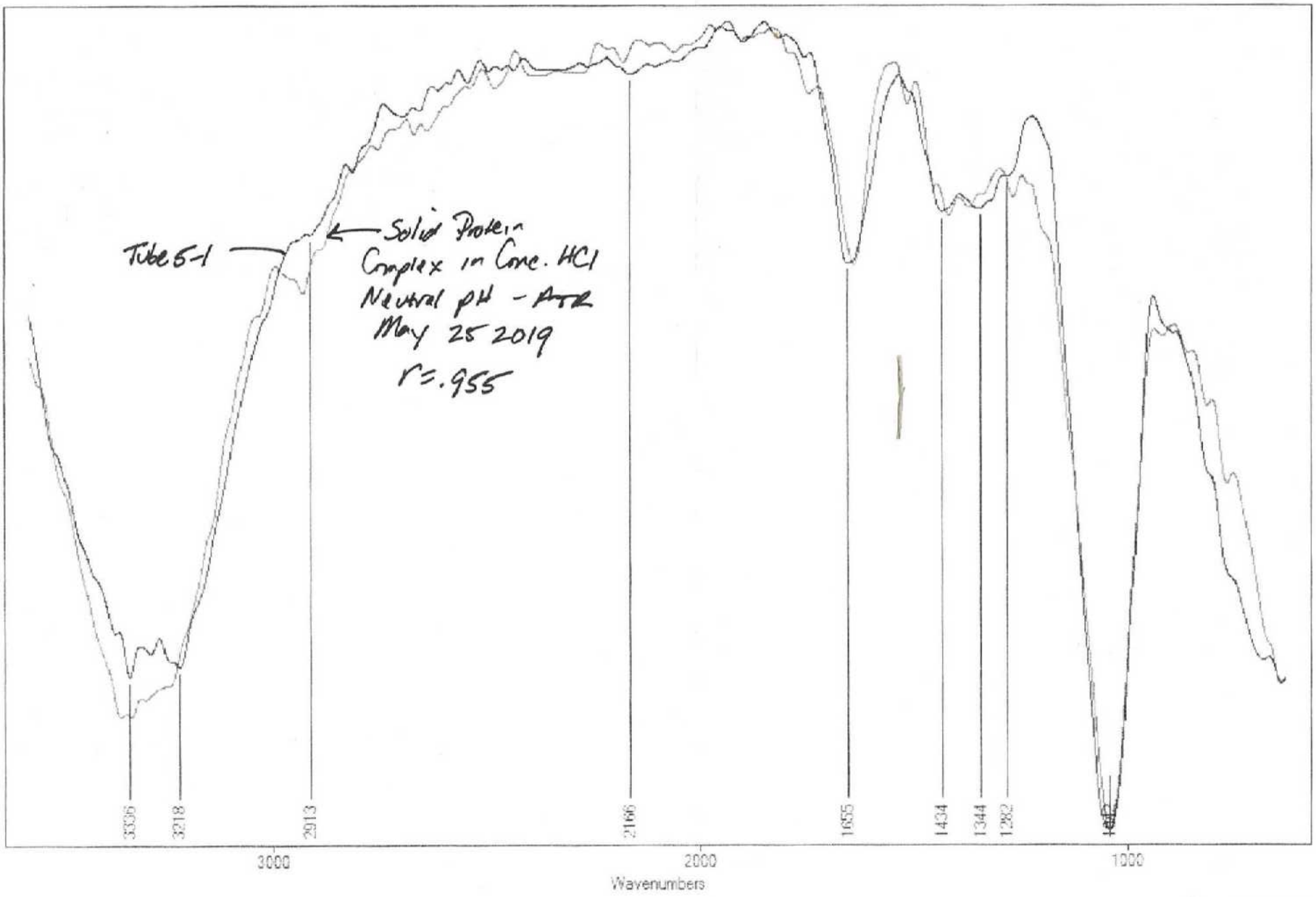
printed on 06/1

CDB Soluble Protein - LC Separation - Tube 5-1 - Jun 12 2019 - 07.spc: 06/13/2019 11:52:35 title



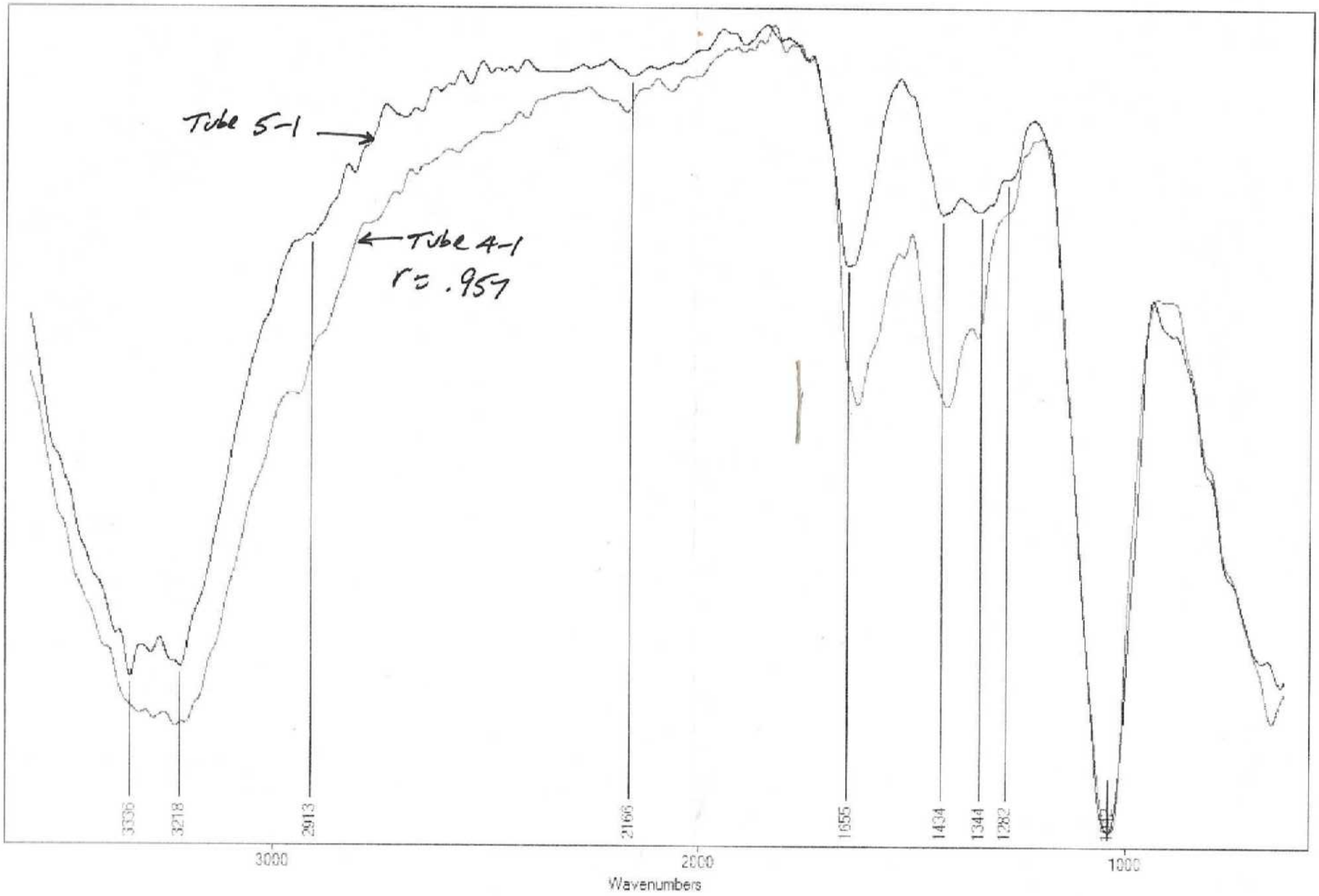
printed on 06/13/2019 12:04:50

CDB Soluble Protein - LC Separation - Tube 5-1 - Jun 12 2019 - 07.spc: 06/13/2019 11:52:35 title



printed on 06/13/2019 12:07:21

CEB Soluble Protein - LC Separation - Tube 5-1 - Jun 12 2019 - 07.spc: 06/13/2019 11:52:35 title



printed on 06/13/2019 12:05:43

June 14 2019

What I wished to do here is run a check on Tube 4-1. This has been done w/ Tube 5-1 as shown on spectra of the previous page.

Result: they are the same.

What is interesting about the result is the following:

We see that the first elution from the soluble protein complex (using H_2O as the elluent) is the same as

the solid protein complex as the the solid complex has been dissolved in Concentrated HCl and subsequently neutralized for preparation on the ATR plate for IR analysis.

There is an interesting level of equivalence between the solid & soluble protein complexes of the CDB culture.

One side of the picture is only discovered by liquid Chromatography elution of a soluble complex (which, incidentally, is already of an acidic nature) with the highly polar eluent of water. It is only the first eluate(s) of the reverse LC run that produces the compound.

The second part of the picture is that of a solid protein complex that is dissolved ~~(at least)~~ into a concentrated mineral acid HCl. This is then neutralized to become only slightly acidic which retains the material as dissolved but allows it to be used on the ATR plate for IR.

Two common grounds lie, therefore, between the solid & liquid protein complexes are that of pH (acidic) and polarity (water soluble) and Protein Positive Colorimetric test results in both cases.

Therefore: Acid, Polar, Protein

Tubes 4-1, 5-1 = Dissolved Solid in HCl

Tubes 4-1 and 5-1 both pass the protein Colorimetric test by giving color.

Protein Reagent Radical Improvement:

The protein reagent has now been vastly improved.

Use RITE Cherry Red Dye

1. Stock solution of red dye is now

300 ul of red dye in 10 ml of H_2O

If raw dye is too thick for pipette add only 1 drop of H_2O to allow for sufficient pipette flow.

Now you need

1. Only 2 ml of H_2O is sufficient - do not go more than 3 ml
2. 100 ul of stock red dye
3. 1 drop 10M NaOH
4. 2 drops 0.5M $CuSO_4$
5. Pinch tartaric

The creates a good steady blue complex (no purple tint)

A Positive test now turns to green (olive green) even more pronounced than the purple before. The reagent is even more sensitive.

Next I would like to start looking @ the titration extraction: Our reference tubes are threefold:

1. Tube 5-1
2. Tube 4-1
3. Solid complex dissolved in Conc. HCl.

What I am especially interested in is the ORP of each of these separations. Since by IR analysis they appear to be essentially the same compound (or might think the ORP of each sample would be similar, & do not think that this is actually the case. Let's see

	ORP	pH	
Tube 5-1	+106	5.67	$\overline{pH} = 6.6$
Tube 4-1 (Tube 4-2)	-130	7.6	

May 25 HCl Dissolved HCl
Solid Complex Sample
This is in Vol 24
You did not have a title here. You used the raw sample dissolved in HCl, pH neutralized for use in ATR plate. We still have some of this left.

Ok, the pH is now neutralized on the dissolved HCl sample.

Neutralized Conc. HCl Sample	+106	6.6

$\overline{ORP} = +54$

Likely Tube 4-1 is the odd one out.

Surely glad for the moment (we are headed towards titration @ some point) & I've received my first EDTA Chemical package, 1 LB.

The is an interesting compound w/ many applications

We have Calcium Disodium EDTA

MW = 374.27 gms/mol

1M solution = $\frac{374.27 \text{ gms}}{1000 \text{ ml}}$ = $\frac{37.43}{100 \text{ ml}}$ = $\frac{22.46}{60 \text{ ml}}$

and a 0.1M solution = ~~0.226 gms~~ $\frac{2.25 \text{ gms}}{60 \text{ ml}}$

While I am @ it I need to prepare 1M NaOH.

$\frac{40 \text{ gms}}{1 \text{ mol}}$ = $\frac{40 \text{ gms}}{1000 \text{ ml}}$ = $\frac{x}{60 \text{ ml}}$ $x = 2.4 \text{ gms}$

but we need 1M = $\frac{24.00 \text{ gms}}{60 \text{ ml}}$

Done.

Back to ORP: Then really is no base for titration if sample very lutinous protein and negative ORP.

We know that no significant titration can occur w/ the raw soluble protein complex and it is how the existence of sulfuric acid was discovered.

Titrations are going to be quite interesting to develop. I have an excellent reference (Analytical Chemistry, Christian, 1971). He covers all four types
1. Acid-base
2. Precipitation
3. Complexometric
4. Redox

An observation that catches our attention is that the soluble protein produces an insoluble complex (Fe^{+3}) with a change of pH to alkaline. Conversely, the solid complex is able to dissolve the complex with a shift to the acid side. We now also know, from LC work, that the solid form share common ground w/ the soluble form.

Ie, the solid complex dissolved in strong HCl appears to match the highly polar front elution from the soluble complex.

Our titration project will now be acetic acid + NaOH

$HC_2H_3O_2 + NaOH \rightarrow NaC_2H_3O_2 + H_2O$

Acetic acid — Sodium Hydroxide — Sodium Acetate + water

Let's prepare a 1.0M solution of NaOH.
40gms
liter.

Let's use $\frac{20gms}{500ml}$ Let's use 0.5M = $\frac{10gms}{500ml}$

So we have a
1. 500ml of 0.5M NaOH solution
2. ~ 500 ml of Vinegar (~ 5% acetic acid)

Now an important question is what type of probes can be used to titrate. Prefer to use pH probe or ORP if possible.

Seems to me that a pH meter should work because we are neutralizing an acid w/ a base. But are looking for a precipitate? (NO!)

Question: Is sodium acetate soluble?
Yes it is. ~ 33gms/liter. So the ~~Change the picture some.~~ will not be able to see the reaction take place.

Beaker wt: 103.50gms
Mass w/ Vinegar: 132.88gms Δ = 29.38gms

I theoretically measured 25.0ml of vinegar.

I find that vinegar has a density of 1.05gms/cm³

Then means that our volume introduced into the beaker is 29.30 gms / 1.05 gms/ml = 27.90 ml However, presumably we measured 25.0 ml w/ a graduated syringe. That is way too much error. = 12% error.

As expected, therefore, we find that measurement of the mass of the volume of sample is far more accurate (assuming we know or can easily to assume the density) than measurement of a volume or graduated volume container.

Often we will assume water when the concentrations are modest. In the case an assumption of water would have led to an error of:

$$\frac{27.90\text{ gms} - 29.30\text{ gms}}{27.90\text{ gms}} = 5\%\text{ error}$$

which is still larger than desired but still a major improvement.

The reason is get the density of the sample as at all possible.

So what we have in the trial is 27.90 ml of vinegar of unknown concentration to be titrated w/ ~~1.00M~~ 0.5M NaOH

How to use the Semi-Auto Titrator:

Our next steps are to control evaporation and to prime the titrant pipette w/ NaOH

We have been done. pH of our initial solution (analyte) of vinegar is ~ 4.02

n=0 pH = 4.02 Setting is Fast, Magnetic Stirrer

i =				
1	Vol = 12.0 ml	pH = 4.87	n = 46	(= 0.261 ml/count
2	Vol = 12.0 ml	pH = 5.13	n = 47	(= 0.255 ml/count
3	Vol = 12.0 ml	pH = 5.36	n = 47	(= 0.255 ml/count)
4	Vol = 12.0 ml	pH = 5.74	n = 47	
5	Vol = 12.0 ml	pH = 9.87	n = 47	
	Σ = 60.0 ml		Σ = 234	$\bar{X}$ = 0.256 ml/count

Flush the pipette burette after completion

The equivalence point has been reached. This shows us, once again, that vinegar is actually a rather strong acid.

We have produced Sodium Acetate here.

Equivalence reached @

n = 29 pH = 6.47 $\bar{X}$ = 29.5 pH = 6.56

n = 30 pH = 6.66

Therefore 29.5 (0.256 ml/count) = 7.55 ml

Equivalence @ 4 (12.0 ml) + 7.55 ml = 55.55 ml @ pH = 6.56

Save it. This is the data required.

1.0 M NaOH would be preferable to reduce the volume by ~ 1/2.

Tomorrow we can analyze the data. Slight Cloudiness Visible.

June 15 2019

It can be seen now that Vinegar is actually quite a strong acid. With our existing NaOH concentration of 0.5M, it is preferable to reduce the concentration of the Vinegar by ~1/2.

Our reaction in this case is known (usually not) as:

$HC_2H_3O_2 + NaOH \Rightarrow NaC_2H_3O_2 + H_2O$

The reaction is now balanced.
EBAS software performs Concentration Calculation easily on NaOH
Conc. = 0.5M and n = 0.25 moles mass = 10gms V = 500ml
Now to BATE-DH analysis software.

$M_1V_1 = M_2V_2$ $\quad\quad$ $\%V_1 = \%V_2$

~~$M_1V_1 = M_2V_2$~~ $\quad\quad$ $C_1V_1 = C_2V_2$

$M_1(55.55ml) = (10gms)(27.98ml)$

~~$M_1 = 5.031gms$~~

~~EBAS gives Concentration of Acetic acid @ 1.51M~~

Let's check this

MW of acetic acid = 60.05 gms/mole $\quad$ 1M solution

$\frac{60.05gms}{1000ml} = \frac{x}{55.55ml}$ $\quad$ $x = 3.34gms$ for 1M solution

but we have $\frac{5.031gms}{3.34gms} = 1.51M$ solution of acetic acid

This really is quite strong. and this is Wrong.

Ok, not ok yet, the known concentration must be in either Molarity or %.

$M_1V_1 = M_2V_2$

[0.5M NaOH] [Vinegar]

$M_1(55.55\,ml) = M_2(27.98\,ml)$

We know the molarity of M_1 (ie 0.5M NaOH) EBAS also gave us this.

Therefore

$0.5M(55.55ml) = M_2(27.98ml)$

$M_2 = 0.993M$ acetic acid.

The looks more reasonable.

Now acetic acid has a MW of $\frac{60.05\,gms}{mole}$

Density = $1.05\,gms/Cm^3$.

$\frac{60.05\,gms}{1000\,ml} \times \frac{X}{1000} (.993) = \frac{59.61\,gms}{1000\,ml\,H_2O} = 5.96\%$

The says our vinegar is ~ 6% concentration and that it is essentially a 1M solution of acetic acid.

The numbers do, be reasonable.

Commercial vinegar is stated most commonly to be @ a concentration level of 5%.

So we are right in range. I accept the titration as valid. Now let's look @ pH behavior.

Note also that we are essentially @ a 1M solution of acetic acid, which may not exactly be a coincidence.

Let's look @ this in more detail. We established our equivalence point @ pH ≅ 6.56

Bate software shows the pH @ our concentration level to be @ 4.76. Bate also seems to depict the equivalence point @ pH of 9.05.
Let's seek an explanation of our difference, the titration seemed quite smooth.

Also notice the pKa of acetic acid is given as 4.76 You did some clever work earlier in identifying an unknown acid. We are headed to that again.

Let's repeat our work w/ a dilution of the vinegar.

Mass of vinegar container = 400.05 gms
Mass of current vinegar = 447 gms.
Diluted by 1/2 to 894 gms. Done

Lets now titrate again & reduced vinegar concentration.

Our density of vinegar is now 1.025 gms/cm3 or 1.025 gms/ml.

Use ~25 ml of the diluted vinegar as the analyte. We have 25.02 ml
Then mean we have 25.02 ml / 1.025 gms/ml = 24.39 gms of dilute vinegar.

Initial pH = 4.09

V = 12.0 ml	pH = 5.37	n = 52	
V = 12.0 ml	pH = 6.83	n = 52	(.231 ml/n)
V = 2.4 ml	pH = 10.20	n = 7	(.343 ml/n)

Equivalence pt @
n = 3 pH = 7.04 $\bar{n}$ = 3.5 $\bar{pH}$ = 7.79
n = 4 pH = 8.54

Volume = (24 ml) + 3.5(.343 ml) = 25.20 ml

Then is the data required.

$M_1V_1 = M_2V_2$

Acetic Acid	NaOH	
V_1 = 25.02 ml	V_2 = 25.20 ml	(amazingly close)
M_1 = ?	M_2 = 0.5M	

M_1 = 0.504 molarity of acetic acid w/ in distilled vinegar.

As we see, we got exactly the same result. Everything says that the vinegar is 1M acetic acid.

Notice our equivalence pt pH is now high, however @ pH = 7.79 vs 6.56

We have a very good titration solution therefore & highly consistent.

Now the question is, how would we go about identifying the unknown acid, in this case acetic acid.

Let's look @ the pKa - equivalence point relationship. Remember that we would not know anything about the unknown acid, including molecular weight.

Here is the relationship:

At the HALF equivalence point, the pH = pKa.

At exactly 1/2 of the volume of the equivalence point, the measured pH is equal to pKa.

Obviously, a very important relationship. Let us see what this means and how to interpret and use it.

Exactly what volume is involved here?

* The volume being referred to is the volume of the titrand that has been added.

So in our case, 25.20 ml of 0.5M NaOH was added.

The half equivalence point, therefore, occurs @ a titrand volume of 12.6 ml. It is @ this volume that pH = pKa.

Therefore: 25.02 ml of ~~acc~~ of 0.504 M acetic acid is reacted w/ 12.60 ml of 0.5M NaOH

Bate will give us that computed pH.

The computed pH is 6.85 :

~~Heat~~ The database record of the pKa for pure acetic acid is 4.76.

Is the pKa of a dilute acid the same as the pH of a concentrated acid?

Note: pKa is another way to express the strength of an acid. A weak acid can actually have a lower pH than a diluted strong acid.

Recalling, in our work w/ identifying an unknown acid, we were dealing w/ Ka, not pKa. Jan 07 2019

Here is an observation. We do indeed compute an equivalence point of pH 9.01 @ our equivalence point. Recall that we measured, however, pH = 7.79.

Ok, here is the method to identify an acid:

1. Compute the pH @ the half equivalence point.

2. At this point, pH = pKa

3. Now compute Ka $= 10^{-pKa}$
(Dissociation Constant)
Polyprotic acids will have multiple Ka's.

4. Identify the acid from a table of Ka's (monoprotic or polyprotic accordingly)

This was done on Jan 15, 2019 (Volume 24)

In our case, $10^{-6.85} = 1.4E-7$

The actual Ka for acetic acid is $1.7E-5$

You also did some work w/ molecular weight determination, which is interesting.

I must have had a table of Ka's for various acids.

Ok, let's run some & see:

1M Acetic acid has a pH of 2.4

Which is also the pH of vinegar that is about 5% acetic acid

Now remember our starting pH ?!!!
It was @ 4.09

So the main value our error is coming from. pH meter must be calibrated!

We are 4.09 − 2.4 = 1.7 pH units too high

Then means our measured equivalence point of (7.19 + 6.56)/2 for mean is
7.2 − 1.7 = 5.5 is actually our equivalence pt

~~and p~~

Ok, Time to Stop the Press!

Notice that functional groups also have pKa's!

The titration is bringing up the importance of pH meter Calibration and the issues of buffers.

At least get 1 pt pH Calibration in place.

One of the issues here is that the titrator is especially demanding and restrictive on what pH can be used for the Calibration process

pH 6.8 buffer in the single point mode

Now, testing the phosphate buffer you had already made, it came out perfectly @ pH 6.87. You could not ask for anything better than this.

			Actual
	Bleach ~~12.5~~	12.6	9.57
	Baking soda ~~8.5~~	8.3	7.54
	Vinegar ~~3~~	2.4	~~3.99~~ 3.98
	Ammonia ~~11~~	11.6	10.48
#1	Buffer as accurate @ 6.86		6.86
#2	Buffer Theory	9.2	8.11

Phosphate Buffer - theory @ 9.2 measured @ 8.11
Baking soda measured 7.54 vs 8.3

Conc.		
HCl	3.1	1.60

Now you understand why you have so much difficulty w/ absolute values of pH.

It is possible that our electrode has inherent instability. Either way, let us form our own pH calibration curve.

	Y Theory	X Mean
Bleach	12.6	9.51
Baby Soda	8.3	7.54
Vinegar	2.4	3.96
Ammonia	11.6	10.48
HCl	3.1	1.60

pH Theoretical = ~~1.89~~ 1.19 (Measured pH) − 0.27

$r = 0.94$

$r^2 = 0.89$

So you will need to use this, and it will represent an improvement.

For instance, our most recent equivalence point for the Acetic Acid - NaOH titration is now closer pH 7.79

It is: ~~1.80~~ 1.19 (7.79) − 0.27 = 9.0

And guess what, the theoretical value, as determined by the BATE software is

9.01

Ok, this is great. We now have a pH calibration curve that should generally equate an improvement.

The calibration curve is now posted on the instrument. Now we have an equivalent point that models theory on our current titration.

Now let's go back to pKa.

We see that in the future we will need to save our data or will need to interpolate w/ some error allotted for.

Example. Our ½ equivalence point occurs w/ a volume of 12.6 ml. We will no longer have the data set for that point. You will be required to interpolate between 12 ml intervals.

$\frac{0.6\ ml}{12\ ml} = 5E-2 \quad 4 + 5E-2(6.83-5.31) = 7.3E-2$

Therefore our ½ equivalence point occurs at

$pH = 5.37 + 7.3E-2 = 5.40$

This means our Computed Ka is now $10^{-5.40} = 4E-6$ vs $1.7E-5$ (theory) or .000004 vs .000017 Calc

They are only off by < ½ of an order of magnitude.

This is quite an range now. Now we can use a pKa - Ka table appropriately.

I see we can also use BATE to GRAPHICALLY determine the half equivalent point w/ very high accuracy. Simply pick halfway on the X axis toward the equivalence point & read the y scale for the pH.

In the case of this titration we do get ~ 4.8 & $10^{-4.8} = 1.6E-5$
Vs our measured pH 5.40 ~ $10^{-5.40} = 4.0E-6$

Remember we do not know the acid or even the reaction involved so we are definitely trying to back out the unknown.

Now you are doing well. You are to consult a table of Ka's (pKa's)

Monoprotic vs Polyprotic is also an important separator.

You will need a pKa - Ka table for Mass also.

Acid pka - Ka Table

Relating pH, Ka, and pKa

pH, Ka, and pKa are all related to each other. For an acid HA:

$K_a = [H^+][A^-] / [HA]$

$pK_a = - \log K_a$

$pH = - \log([H^+])$

At the halfway point on an equivalence curve, $pH = pK_a$

Ka of Weak Acids

Name	Formula	K_a	pK_a
acetic	$HC_2H_3O_2$	1.8×10^{-5}	4.7
ascorbic (I)	$H_2C_6H_6O_6$	7.9×10^{-5}	4.1
ascorbic (II)	$HC_6H_6O_6^-$	1.6×10^{-12}	11.8
benzoic	$HC_7H_5O_2$	6.4×10^{-5}	4.2
boric (I)	H_3BO_3	5.4×10^{-10}	9.3
boric (II)	$H_2BO_3^-$	1.8×10^{-13}	12.7
boric (III)	HBO_3^{2-}	1.6×10^{-14}	13.8
carbonic (I)	H_2CO_3	4.5×10^{-7}	6.3
carbonic (II)	HCO_3^-	4.7×10^{-11}	10.3
citric (I)	$H_3C_6H_5O_7$	3.2×10^{-7}	6.5
citric (II)	$H_2C_6H_5O_7^-$	1.7×10^{5}	4.8
citric (III)	$HC_6H_5O_7^{2-}$	4.1×10^{-7}	6.4
formic	$HCHO_2$	1.8×10^{-4}	3.7
hydrazidic	HN_3	1.9×10^{-5}	4.7
hydrocyanic	HCN	6.2×10^{-10}	9.2
hydrofluoric	HF	6.3×10^{-4}	3.2
hydrogen peroxide	H_2O_2	2.4×10^{-12}	11.6
hydrogen sulfate ion	HSO_4^-	1.2×10^{-2}	1.9
hypochlorous	HOCl	3.5×10^{-8}	7.5
lactic	$HC_3H_5O_3$	8.3×10^{-4}	3.1

nitrous	HNO_2	4.0×10^{-4}	3.4
oxalic (I)	$H_2C_2O_4$	5.8×10^{-2}	1.2
oxalic (II)	$HC_2O_4^-$	6.5×10^{-5}	4.2
phenol	HOC_6H_5	1.6×10^{-10}	9.8
propanic	$HC_3H_5O_2$	1.3×10^{-5}	4.9
sulfurous (I)	H_2SO_3	1.4×10^{-2}	1.85
sulfurous (II)	HSO_3^-	6.3×10^{-8}	7.2
uric	$HC_5H_3N_4O_3$	1.3×10^{-4}	3.9

Now we are in a position to examine our table for candidate matches.

We can also use the Bate software as an alternative.

Our calculated value for Ka is ~ 4.0E-6

Acetic acid : $\sim 10^{-5}$
~~Carbonic acid : $\sim 10^{-6}$~~ polyprotic
hydrazoic acid $\sim 10^{-5}$

Well guess what, our closest match to any monoprotic acid is acetic acid

$pKa = 4.76$ or $Ka = 10^{-4.76} = 1.7E-5$

off by a ratio of 4.3
That is less than ½ of an order of magnitude.

Ok, great work so far. Here.

Now that we are in place again, we should revisit the soluble protein fractionation w/ the newer instrument.

—

I have made a custom pipette tube to be used to determine the density of a small volume of liquid, ie, ~ 3 mls. It should work.

Our current project is to determine the density of the soluble protein complex.

Mass of tube alone: ~~9.51 gms~~ ~~9.55 gms~~ 9.559 gms ~~9.54 gms~~

Mass w/ protein filled to 1.0 mark: ~~10.76 gms~~ 10.74 gms

Mass w/ protein filled to 4.1 mark 14.79 gms

Therefore: $\frac{(14.79 - 10.74)}{3.10\ ml} = \frac{4.05\ gms}{3.10\ ml} = \frac{x}{1.00\ ml}$ $x = 1.31$

So the density of our soluble protein complex is currently @ 1.31 gms/ml.
OK, this worked really well.
We know that if it were pure water it would be 1.00 gms/ml. This tells us that we have 0.31 gms of complex per ml. It also tells us that the solution should be $100 - \left[\frac{1.31 - 1.00}{1.31}\right] = 76.3\%$ water.

Now we can weigh the protein and we know how much of the soluble protein we have in the titrant analyte sample.

We have 4.23 gms dissolved in a total volume of 25.02 gms H_2O

Initial pH of soluble dilute protein = 3.99

Vol = 12 ml pH = 9.74 n = 53

We have almost an immediate equivalent point
n = 5 pH = 4.18 We know that our volume here ≈ (0.343 ml/count
n = 8 pH = 6.19 ~~Estimate ≈ 0.32 ml/count~~
$\bar{X}$ = n = 6.5 $\overline{pH}$ = 5.18
Therefore Volume = 6.5 (0.32 ml/count / .343) = ~~2.08 ml~~ 2.23 ml

This is our first equivalence point

We have a second equivalence point @:

n = 37 pH = 8.49
n = 38 pH = 8.72
$\bar{n}$ = 37.5 $\overline{pH}$ = 8.60

(37.5)(.26 ml/count) = 9.98 ml

Our flow rate estimate
or Volume Flow Ratio = .343
n = 1 ~~Flow~~ = 2.23 ml/~~count~~
n = 31.5 Flow Rate = .266 ml/count
n = 53 Flow Rate = .226 ml/count

We have two equivalence points. The first one occurs @ pH = 5.18 w/ a volume of 2.23 ml

Our second equivalent point occurs @ pH = 8.60. w/a volume of 9.98 ml

Notice the solution turned dark green immediately near the first Equivalence point.

Now, our half equivalent points occur @ 2.23/2 = 1.15 ml

Our second Equiv Pt occurs @ 9.98/2 = 4.99 ml

Now we still have the dark count available so

$\frac{1.15\ ml}{.343\ ml/count} \Rightarrow n = 3.3$

The pH here is 4.00

The 2nd half equivalence occurs @ 4.99 ml $\Rightarrow n \cong 16$
$\sim \left(\frac{0.304\ ml}{count}\right)$

and the pH here @ n=16 is 7.20

So our two Ka estimates are $10^{-4} = 1E-4$ See Next page
for a diprotic acid. $10^{-7.20} = 6.3E-8$

Find these Ka's for a diprotic acid.

We forgot a step here.
We must adjust our pH values in accordance w/ the Calibration Curve

$pH = 1.19(\text{Meas pH}) - 0.27$

Our first EP (equiv pt) measured @ 5.18 pH
Actual $pH = 1.19(5.18) - .27 = 5.89$

Our second EP measured pH 8.60
Actual $pH = 1.19(8.60) - .27 = 9.96$

Now our first 1/2 EP actual pH is
$1.19(4.00) - .27 = 4.49$

So $Ka_1 = 10^{-4.49} =$ ~~3.09E-4~~ $3.24E-5$

Our 2nd 1/2 EP actual pH is:
$1.19(7.20) - .27 = 8.30$

so $Ka_2 = 10^{-8.30} = 5.01E-9$

Our strongest candidates here are

	Ka_1	Ka_2
Sulfurous Acid (Not Sulfuric)	1.54E-2	1.23E-7
Phosphoric Acid	7.11E-3	6.32E-8

*

Alternate Table: pKa & Ka.

Dissociation Constants for Acids at 25 °C.

Name	Formula	K_a (or K_{a1})	K_{a2}	K_{a3}
Acetic	$HC_2H_3O_2$	1.8×10^{-5}		
Arsenic	H_3AsO_4	5.8×10^{-3}	1.1×10^{-7}	3.2×10^{-12}
Arsenous	H_3AsO_3	5.1×10^{-10}		
Ascorbic	$H_2C_6H_6O_6$	8.0×10^{-5}	1.6×10^{-12}	
Benzoic	$HC_7H_5O_2$	6.5×10^{-5}		
Boric	H_3BO_3	5.8×10^{-10}	1.8×10^{-13}	1.6×10^{-14}
Butanoic	$HC_4H_7O_2$	1.5×10^{-5}		
Carbonic	H_2CO_3	4.3×10^{-7}	5.6×10^{-11}	
Chloroacetic	$HC_2H_2O_2Cl$	1.4×10^{-3}		
Chlorous	$HClO_2$	1.1×10^{-2}		
Citric	$H_3C_6H_5O_7$	7.4×10^{-4}	1.7×10^{-5}	4.0×10^{-7}
Cyanic	HCNO	3.5×10^{-4}		
Dichloroacetic	$HC_2HO_2Cl_2$	5.0×10^{-2}		
Formic	$HCHO_2$	1.8×10^{-4}		
Hydroazoic	HN_3	1.9×10^{-5}		
Hydrocyanic	HCN	6.2×10^{-10}		
Hydrofluoric	HF	6.8×10^{-4}		
Hydrosulfuric	H_2S	5.7×10^{-8}	1.3×10^{-13}	
Hypobromous	HBrO	2.3×10^{-9}		
Hypochlorous	HClO	3.0×10^{-8}		
Hypoiodous	HIO	2.3×10^{-11}		
Iodic	HIO_3	1.7×10^{-1}		
Lactic	$HC_3H_5O_3$	1.4×10^{-4}		
Malonic	$H_2C_3H_2O_4$	1.4×10^{-3}	2.0×10^{-6}	
Nitrous	HNO_2	4.5×10^{-4}		
Oxalic	$H_2C_2O_4$	5.6×10^{-2}	5.4×10^{-5}	
Phenol	HC_6H_5O	1.3×10^{-10}		
Phosphoric	H_3PO_4	7.5×10^{-3}	6.2×10^{-8}	4.2×10^{-13}
Propionic	$HC_3H_5O_2$	1.3×10^{-5}		
Pyruvic	$HC_3H_3O_3$	2.8×10^{-3}		
Selenous	H_2SeO_3	2.3×10^{-3}	5.3×10^{-9}	
Sulfuric	H_2SO_4	Large	1.2×10^{-2}	
Sulfurous	H_2SO_3	1.2×10^{-2}	6.6×10^{-8}	
Tartaric	$H_2C_4H_4O_6$	9.2×10^{-4}	4.3×10^{-5}	
Thiocyanic	HSCN	1.3×10^{-1}		
Trichloroacetic	$HC_2O_2Cl_3$	2.2×10^{-1}		

Dissociation Constants for Bases at 25 °C.

Name	Formula	K_b
Ammonia	NH_3	1.8×10^{-5}
Aniline	$C_6H_5NH_2$	4.3×10^{-10}
Dimethylamine	$(CH_3)_2NH$	5.4×10^{-4}
Ethylamine	$C_2H_5NH_2$	6.4×10^{-4}
Hydrazine	NH_2NH_2	1.3×10^{-6}
Hydroxylamine	$OHNH_2$	1.1×10^{-8}
Methylamine	CH_3NH_2	4.4×10^{-4}
Pyridine	C_5H_5N	1.7×10^{-9}
Trimethylamine	$(CH_3)_3N$	6.4×10^{-5}

Here are some errors that have arisen in the titrations @ the early stages.

1. All measured pH values must be adjusted for calibration of identification as an error.

2. You also need to calculate the flow rate as it is affected by gravity.

3. Regarding the evaluation of phosphoric & sulfurous acid candidates:

   a) Acid was produced prior to the use of phosphorous in the cultures, thus casting the vote toward sulfurous acid.

   b) What is the difference between sulfuric and sulfurous acid? Likelihood of appearance?

   c) Phosphoric acid is a triprotic acid, sulfurous is diprotic. We only have evidence of diprotic thus far and thus too early a void of a sulfurous acid.

   d) Let's look @ error analysis between the two acid candidates.

Especially if Hoffman clamp is disturbed.
Flow Rate/Volume Calibration should be done for each session.
Let's take care of the flow rate calibration once, assuming the Hoffman tubing clamp is not disturbed.

$n=7$ flow rate = 0.343 ml/count
$n=52$ flow rate = 0.231 ml/count

$\text{Flow rate} \cong -2.49E\text{-}3(n) + .36$
and
$\text{Volume} = \text{Flow Rate} \cdot n$

$\text{Volume} \cong n(-2.49E\text{-}3n + .36)$

$\text{Volume} \cong -2.49E\text{-}3n^2 + .36n \quad n \leq 52$ ~~$n \leq 52$ $n \leq 38$~~

and so we see here that the volume is a quadratic function of n.

Now for some analysis of Phosphoric vs sulfurous acid.

			RMS Ratio	log
Sulfurous Acid	$\Delta Ka_1 = 3.24E\text{-}5$ / $1.54E\text{-}2$	ΔKa_2 1.23E-1 / 5.01E-9		
	Ratio = 415	Ratio = 246	535	2.73
Phosphoric Acid	$\Delta Ka_1 = 7.11E\text{-}3$ / $3.24E\text{-}5$	ΔKa_2 : 6.32E-8 / 5.01E-9	253	2.4
	Ratio = 219	126		

This means that between the two, phosphoric acid is actually the stronger candidate. Log of the ratio error is the best candidate

The says, if possible, you would like to develop a test that will distinguish between phosphorous or sulfurous acid.

In the meantime, lets see if we can discern a third pKa.

$10^{-pKa} = 4.467E-13$ $\quad pKa = 12.3$

This will be very difficult to detect but we can try.

Indeed, the titration curve plot shows that it is not possible.

Let's inspect the titration curve and see if we can find a difference.

With our respective concentrations we established the following two equivalence points:

pH 5.09 We use 12.0 ml of 0.5 NaOH
pH 9.96

We need to balance the equation between H_3PO_4 & NaOH.

$H_3PO_4 + NaOH \rightarrow H_2O + Na_3PO_4$

to balance this we have (Ebas software check)

$H_3PO_4 + 3NaOH = NaPO_4 + 3H_2O$

This tells us that one mole of H_3PO_4 reacts w/ 3 moles of NaOH

We see that for our NaOH part of our reaction we are using 6E-3 moles of NaOH. This means that 2E-3 moles of H_3PO_4 would be used.

For the unknown analyte (assuming it might be phosphoric acid) we used a total volume of ~25.02 mL

Fortunately the titration curve does not change very much as long as we have the number of moles specified! The BATE (pH) software shows us three titration equivalence points

pH = 4.69 5.89 (meas) Δ = 1.20
pH = 9.89 vs 9.96 (meas) Δ = .07
pH = 12.34

Now we do the same for sulfurous acid; ie, expect the titration curve.

Our reaction for sulfurous acid is
sodium sulfite

$$H_2SO_3 + NaOH \rightarrow Na_2SO_3 + H_2O$$

Balanced goes to:

$$H_2SO_3 + 2NaOH = Na_2SO_3 + 2H_2O$$

This means 2 moles of NaOH are used for every mole of sulfurous acid.

We have 6E-3 moles of NaOH
Therefore we use 3E-3 moles of sulfurous acid.

The titration curve shows the following two equivalence points.

pH = 4.39	vs	5.89	Δ = 1.50
pH = 9.88		9.96	Δ = .08

* And we see that our differences between these two acids are too close to call.

Also refer back to Notes circa Jan 15 2019
For first (mistaken) attempts at identification.

Sulfurous acid is an unstable weak acid when sulfur dioxide dissolves in water. It is used as a reducing and bleaching agent.

It is an "elusive acid". Pure anhydrous sulfurous acid has never been isolated or detected.

* Everything now says, therefore, that we are dealing with

PHOSPHORIC ACID in the soluble protein
* complex, NOT "SULFUROUS ACID".

There is an extremely important distinction.
The notes of Jan 2019 must now
* reflect the latest determination.

The notes of Jan - Feb 2019 are
* now reflected, at least partially,
to show the most recent work.

Recall also the notes of Feb 05 2019
also make reference to the positive
detection of phosphate ions.

The Hazards of Phosphoric Acid
Major Consequences Here

And now for the big discovery and consequence of the book.

* Guess what is known to dissolve teeth ?????

* Phosphoric acid

Guess why teeth and dental problems are one of the main symptoms showing up in the Mayzell Research Project survey?

* Phosphoric Acid

Phosphoric acid dissolves calcium.

Joint pain.

* Phosphoric Acid

The Hazards of Phosphoric Acid
This all makes sense w/ the symptoms recorded.

Phosphoric Acid (Dr. Axe)

* 1. Lowers Bone Density (teeth, bone, joints)

* 2. Tissues may damage kidney tissues

* 3. Decrease nutrients in the body:

1. Calcium	Remember Mercola sees the deficiencies -
2. Iron	1. Iodine
3. Magnesium	2. Vitamin D
4. Zinc	3. Magnesium

* 4. Increase Body Acidity

* 5. Damages Tissue
(used for rust removal)

Phosphoric Acid is the strongest ~~acid~~ Acid (the lower pka)

→

We see now that the data has already paid itself off many times over and this is only the first project that it has been applied to.

The knowledge of the existence, i.e, dominance of Phosphoric acid is invaluable and it explains many of the symptom reports already known.

We notice that a major dark green complex is formed when we add alkaline to the soluble proteins. What is the complex?
What if you were to separate out and then analyze it?

You also need to NIR analyze any of the LC separations, either from the soluble or insoluble complexes if they formed a film on the TLC plates.

Now I will monitor the formation of the dark green complex.
Slow: ~30 counts/mL

3.04 gms Soluble Complex
25.08 gms total mass w/ H_2O added.

Titrate w/ 0.5M NaOH in SLOW mode.
Initial pH = 4.02 – Slow Mode ≤ 3 mL

The solution turns dark green immediately after the first equivalence point is reached

Vol = 1.6 mL n=48 pH = 7.21 3.33E-2 mL/count

We have an equivalence pt @
n = 37 pH = 4.77
n = 38 pH = 5.41
n = 37.5 pH = 5.09 Volume = 37.5 (3.33E-2 mL/count) = 1.25 mL

Adjusted pH = 1.19(5.09) - .27 = 5.79
Previous measurement was 5.89 so this is excellent
1/2 equivalence pt occurs @ 0.625 mL
n = .625 mL / (3.33E-2 mL/count) = 18.8 ≅ 19 for a
pH @ n=19 = 4.00
Adjusted pH = 1.19 (4.00) - 0.27) = 4.49 = pKa
and Ka = $10^{-4.49}$ = 3.24E-5
We determined the exact same value last time, 3.24E-5
Published value is 7.11E-3. This tells us our pH meter is reading too high in the lower ranges. But it is acceptable error. Error ratio = 7.11E-3 / 3.24E-5 = 219
This is approx 2 orders of magnitude in the lower pH range.
The actual pKa is ~ 3.3 vs 4.5 – Not too bad.

We now have some form of dark green complex that is formed.

Can we separate this & analyze to some degree?

Separation: YES. I have very successfully centrifuged this dark green complex. It seems as though it may be suited for NIR analysis on an evaporated ceramic plate.

It can also likely be redissolved into an acid environment. Realize that this is still in an acidic state (pH ~ 5.7)

Drained, rinsed & repeat centrifuge.

I now have a very concentrated, dark green, clear solution of the complex formed after the first pKa. I have spotted this on the ceramic plate (~ 1/2" diameter) and it should be suitable for NIR analysis. Currently under evaporation.

It also looks like it will be suitable for mid IR on ATR plate.

We do have an NIR scan.
We show very light absorbance @ 900 nm
but not a singular peak. Recommend that
we also use the UV-VIS-NIR spectrometer
for range from 700 – 1100.

We also have a peak absorbance @ ~ 1442 nm.

General NIR Chart shows ROH, $CONH_2$ & CH
Candidates.

Osborne table shows +/- 10 nm:
NH : $CONH_2$ @ 1430
OH : sucrose, starch @ 1440
CH : @ 1440
CH aromatic : @ 1446
Starch, H_2O (OH) : @ 1450

2nd NIR scan gives:

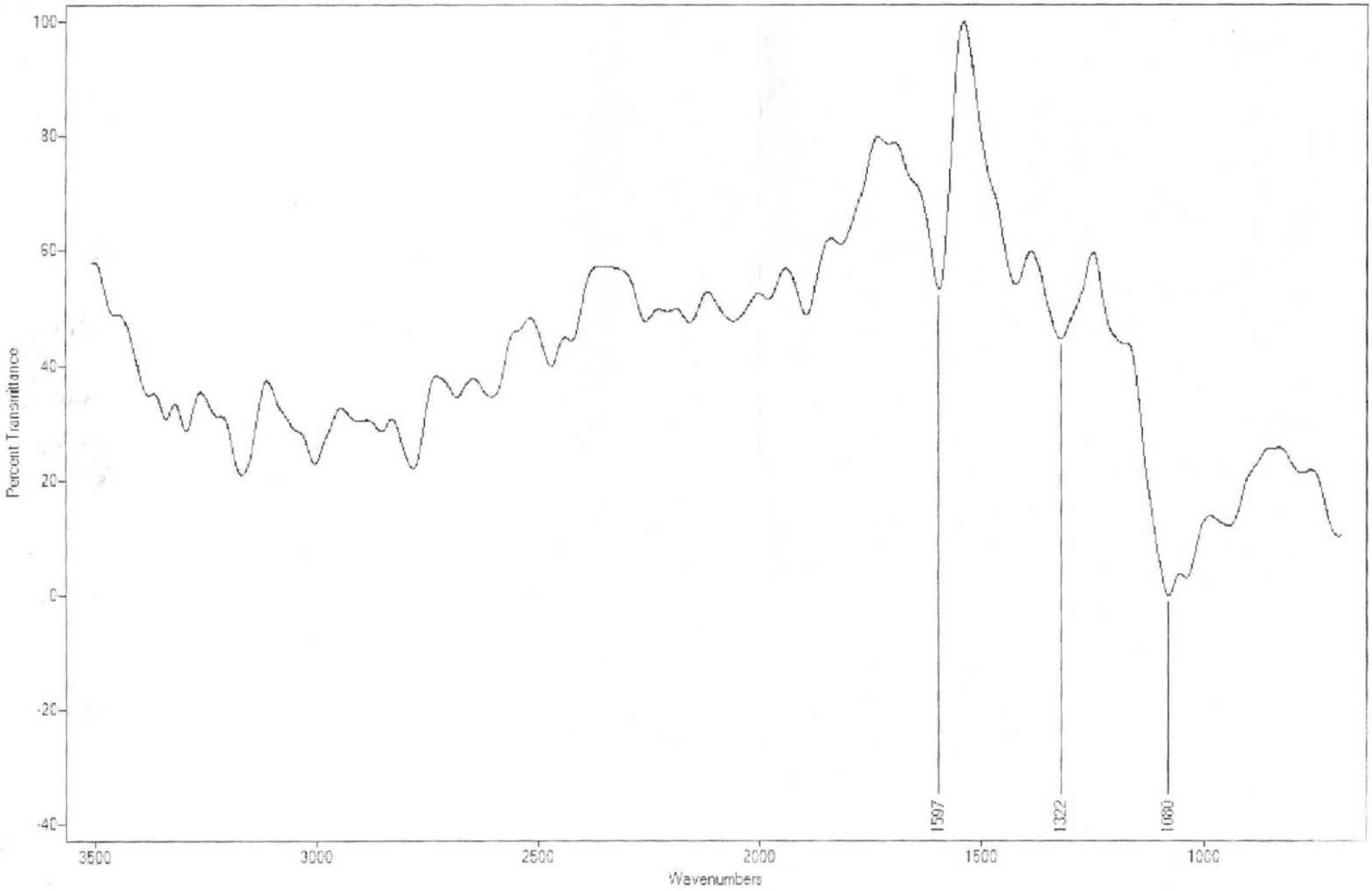
900-904 Very strong CH_3 Not a peak

1444 nm - CH aromatic (from above)

The compound dries to a very fine powder.

Soluble Protein Complex - 1st pKa Titration Complex

CDB Titration - 1st pKa Complex - Dark Green Precipitate - Jun 15 2019 - 01.spc: 06/16/2019 03:01:49 title



eFTIR

printed on 06/16/2019 03:06:06

IR gives very little response. We have 3 peaks only in the fingerprint region.

moderate	1597 cm^{-1}
weak	1322 cm^{-1}
strong	1080 cm^{-1}

1597 cm^{-1} ~1600 C=C
1620 - 1560 NH_2^+
1620 - 1590 N-H
1625 - 1585 C=C aromatic C=C

1322 cm^{-1} strong 1340 - 1280 S=O R_2SO_2 Sulfone

1080 cm^{-1} vy strong 1090 - 1030 P-O-C phosphoric ester
↑ a very strong candidate w/ little to no competition

UV-VIS-NIR 700 - 1100nm
We have a small but definite peak @ 1000nm. This is ArCH w/ not any competition.

Absorption @ 900nm was not a peak, it continues to increase all the way to 700nm.

June 16 2019

Concentrated H_2SO_4 will re-dissolve the dark green complex and turn it back to a yellow color, but moderately strong HCl will not.

This means that it can not be put on to the IR ATR plate in this state.

Thus far our information indicates:

1. ArCH structure
   - nm a) from 1446 NirvaScan NIR
   - nm b) from 1080 UV-Vis-NIR spectrometer
   - nm c) 1591 cm^{-1} mid IR absorption

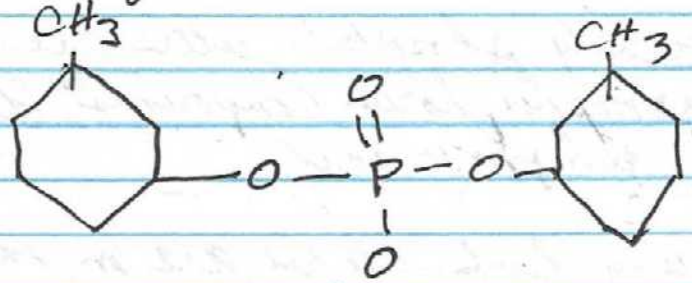
2. Phosphoric ester seems to be a strong candidate from mid IR @ 1080 cm^{-1}

   P-O-C

3. Highly insoluble above pH of 1st pKa (presumably of Phosphoric acid) of pH 5.79 @ equivalence point.

* Well, this is definitely a known related topic.

Aromatic phosphate esters are plasticizers. We have seen the application and topic area before.



Tricresyl Phosphate is apparently a representative example (TCP).

Looks like we have some toxicity issues w/ this class of compounds - related to skin absorption questions, poly neuropathy (not good), liver.

FEP Aromatic Phosphate Esters react w/ metals, especially iron.

Aromatic phosphate esters are typically used in PVC.

These are organophosphate compounds. Remember organophosphates in the rainwater analysis?

Phosphorus & Nitrogen are both in group 15 of the periodic table so they share similar properties.

Technically phosphate esters are not "organophosphorus compounds" but esters of phosphoric acid.

It certainly looks like we are on the right track w/ the second esterification of phosphoric acid.

Malathion, parathion are in the league -ie toxic pesticides

Glyphosate (Roundup) is related here. It is an ester of phosphonic acid instead of phosphoric acid.

Let's look @ the ester test of May 27 2019. Note that the ester is dissolved in ethanol. Our sample is strongly ~~acidic~~ acidic. They want it in an alkaline environment. The should precipitate it, so not sure how the test can proceed. Let's try.

The candidate ester sample turns the reagent orange w/ a precipitate prior to heating, so the control reference has already been lost.

The test was now repeated w/ only 30 ml of the Candidate extract since it seems to be highly concentrated.

It does indeed cause a strong precipitate. Apparently the sample boiled dry. No result available.

ROAD CONSTRUCTION: DETOUR

Let's start looky @ EDTA complexometric titrations

Ferrous sulfate heptahydrate has a MW of 278.01 gms/mole

$.5M = .1M = \frac{27.8 \text{ gms}}{1000 \text{ ml}} = \frac{x}{200 \text{ ml}}$ $x = 5.56$ gms

Ca Disodium EDTA $= \frac{374.27 \text{ gms}}{\text{gms/mol}} \Rightarrow 0.1M = \frac{37.43 \text{ gms}}{1000 \text{ ml}} = \frac{18.72 \text{ gms}}{500 \text{ ml}}$

Keep track of the Fe^{+2} solution above 5.0

These solutions have now been made:
1. 0.1M $FeSO_4$ (200 ml)
2. 0.1M $CaNa_2$ EDTA (500 ml)

Let's try a titration. pH of Fe^{+2} solution should be above 4.0 for titration to occur.

I start w/ 25 ml 0.1M $FeSO_4$.
The pH may be a difficult issue.
Original pH was 4.9. I added one drop
1M HCl and precipitation begins and pH is
now 5.15. I do not know what to expect
here. Use Fast Mode

5.10
Initial pH ~~5.14~~. Slight precipitation. Fast Mode

It looks like the equivalence point was almost
immediately after 5.10 pH immediately rose to
~6.6 and remained stable.

I believe we need to acidify the $FeSO_4$ to pH 3 or 4.

This time I add two drops 1M HCl and
bring the initial pH down to 3.91.
$FeSO_4$ solution is clear.
Initial pH = 3.89

FAST MODE Volume = 12.0 ml pH = 4.57 n = 50
SLOW MODE Never go more than 3 ml in slow mode.
A very clear light green color has formed.

4.90
SLOW Volume = 3.0 ml pH = ~~4.79~~ n = 91
It is said that EDTA is acidic, but
the fact is that it is increasing the pH.

6.42
SLOW Volume = 3.0 ml pH = Still changing = ~~6.35~~ n = 100

Actually you maxed out the data collection. The way you probably should not go more than 2 mL in the slow mode. That will only be for very exacty work.

When you are right now in the equivalence point. Volume = 12.0 + 3.0 + 3.0 mL = 18.0 mL
That is it.

The other thing you notice is that the color development remains clear. No precipitation has occurred like when you added NaOH. Hard to believe we will be able to detect millimole concentration but we will see. You are currently @ 0.1 Molar
You used 5.56 gms in 200 mL
Millimole would be ~0.069 ms / 200 mL = $\frac{1}{x}$
$x = 3330$ mL ~ 1 part in 3300. Not bad.

Let's see you can determine concentration with the current scenario.

One lesson is acidify the Analyte below the minimum pH required (my Irena table is available). It also did form a heavily colored complex.

This was great.

So the big question is, of course - what if you do not know the metal involved?

Jun 17 2019

I would next like to try mercurial Concentration detection. Let's try .05 gms in 200 ml

We currently have $\frac{5.56 \text{ gms}}{200 \text{ ml}} = \frac{.05 \text{ gms}}{x}$ $x = 1.8$ ml

So lets take 2 ml of the current $FeSO_4$ analyte and dissolve it in 198 ml distilled H_2O and see what can be done. - Ok, Done

Now use 25 ml of the stock dilute solution as the analyte and perform the titration. Oddly enough the solution is highly diluted still has significant color to it.

—

Initial pH = 6.61 so certainly at a less concentrated acidity to pH ≈ 4.0 1 drop 10 M HCl is plenty.

Initial pH = 3.90 Solution still has color.

Fast Mode Vol = 6.1 ml pH = 5.44 n=19 (0.32 ml/count)

pH has already changed rapidly to some extent.

Maximum slope @

n = 5 pH = 4.15

n = 6 pH = 4.64

n = 5.5 pH = 4.395 Vol = (5.5)(.32 ml) = 1.76 ml

Adj pH = 1.19(4.395) - 0.27 = 4.96

Let's Continue: pH = 5.45

Vol = 12.0 ml pH = 5.75 n = 40
Vol = 12.0 ml pH = 5.00 n = 40

So this tells us that our equivalence point was already reached very early in the game. It is also of great interest that the solution turned very clear @ this point. So we know that it was plenty sensitive to detect the level of concentration.

Next run will be 5 drops of the original FeSO4 dropped into a total volume of 200 ml & we will attempt another run in SLOW MODE.

We see now that clarity of the solution (Color a otherwise) ie, transparency, is when the reaction is occurring.

Initial pH = 7.12 Concentration is so low that solution is already transparent. Acidify the solution. PH is now 3.09 w/ 1 drop HCl (10M) added.
(We slow mode. (2 ml max)
Initial pH = 3.89
Volume = 2.0 ml pH = 4.92 n = 63 (.032 ml/count)
We have a maximum slope reached:
n = 47 pH = 4.45
n = 40 pH = 4.71
$\bar{n}$ = 41.5 $\overline{pH}$ = 4.58
Volume = 41.5(.032) = 1.52 ml
Notice pH is reasonably close to last value.
Adj pH = 1.19(4.58) − 0.27 = 5.18 Equivalence Point Reached

adj Mean pH from last two points is 5.09 (4.49) 4.395 ~~4.47~~ (~~4.47~~ + 4.58/2)

Acidification of the analyte w/ use of EDTA is first off, some observations. Critical.

The method of titration w/ EDTA is indeed quite sensitive, and that is great. I am definitely able to detect an equivalence point in all cases, even under a rather weak concentration of $FeSO_4$.

Color is no requirement. We do note that the solution actually is clarified @ the equivalence point.

I need to learn next what concentration levels have been detected and if there is any way that EDTA titration methods can be used to IDENTIFY the assay analyte, analogous to what has been accomplished with acid-base titrations.

Concentration issue:

1. Our first run was @ 0.5M $FeSO_4$. No issue there.

2. Next we took 2 ml of 0.5M $FeSO_4$

$.5M\ FeSO_4 \cdot 7H_2O = \frac{5.56\ gms}{200\ ml} = \frac{x}{2\ ml}$ $x = .056\ gms$

The .056 gms is now further dissolved into 200 ml H_2O

Therefore $\frac{.056\text{gms}}{\frac{200\text{ml}}{5.56\text{gm}}} = .01$ Therefore we are dealing w/ a concentration of

$\frac{5.56\text{gm}}{200\text{ml}}$ $.01(0.5\text{M FeSO}_4) = .05\text{M FeSO}_4$

OK, not bad, but we hope for more and we should have it.

3. Next we take 5 drops of the 0.5M $FeSO_4$ solution $\cong$ 5 drops $(.05\text{ml/drop}) \cong 0.25$ ml of 0.5M $FeSO_4$

$\frac{5.56\text{gms}}{200\text{ml}} = \frac{X}{.25\text{ml}}$ $x = 6.95\text{E-}3\text{gms}$

Now we take this 6.95E-3gms and dissolve it into 200ml H_2O

$\frac{6.95\text{E-}3}{\frac{200\text{ml}}{5.56\text{gms}}} = 1.25\text{E-}3$ So our concentration is

$1.25\text{E-}3(0.5\text{M}) = 6.25\text{E-}4\text{M}$ $FeSO_4$

And there is superb. We are past the millimole stage level that we hoped for. Lets look @ parts per thousand.

$6.25\text{E-}4\text{M}\ FeSO_4 = \frac{278.01\text{gms}}{1000\text{ml}}(6.25\text{E-}4) = \frac{0.174\text{gms}}{1000\text{ml}} = \frac{0.174\text{gms}}{1000\text{gms}}$

so:

$\frac{0.174\text{gms}}{1000} = \frac{1}{X}$ $x = 5747$ Parts per thousand.

We now know we are able to detect Fe^{+2} analyte via EDTA titration @ a minimum level of 1 in 5000. It may be that we are able to detect @ an even more sensitive level, ie 1 in 10000. Either way, this is excellent work.

EDTA pH Chart - Analytical Chemistry - Invaluable
Gary Christian

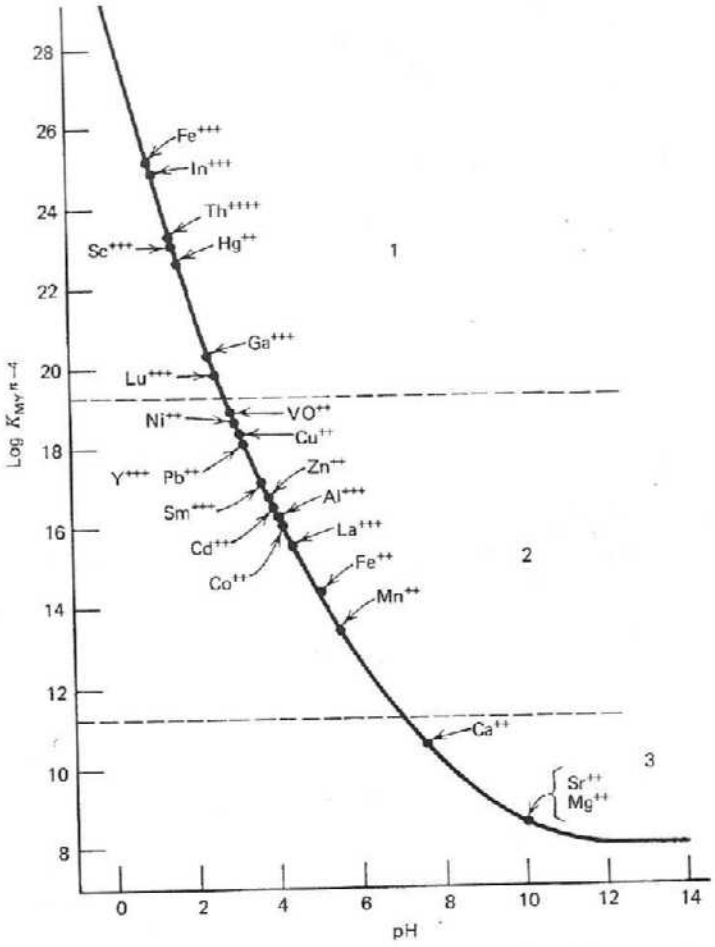


FIGURE 11.3
Minimum pH for effective titration of various metal ions with EDTA. Reprinted with permission from C. N. Reilley and R. W. Schmid, *Anal Chem.*, **30** (1958) 947. Copyright by the American Chemical Society.

Mg^{2+}; the color of the uncomplexed indicator is blue. As soon as all the free Mg^{2+} is titrated, the EDTA displaces the indicator from the magnesium, causing a change in the color from red to blue:

$$\underset{\text{red}}{MgIn^-} + \underset{\text{colorless}}{H_2Y^{2-}} \rightarrow \underset{\text{colorless}}{MgY^{2-}} + \underset{\text{blue}}{HIn^{2-}} + H^+ \qquad (11.15)$$

This will occur over a pMg range and the change will be sharper if the indicator is kept as dilute as possible and still give a good color.

Of course, the metal indicator complex must be less stable than that of the metal-EDTA complex, or else the EDTA will not displace it from the metal. On the other hand, it must not be too weak, or the EDTA will start replacing it at the beginning of the titration, and a diffuse end point will result. In general, the metal indicator complex should be 10-100 times less stable than the metal-titrant complex.

The formatio
close to differer
will titrate toge
titration is used
11.4). Eriochrom
with EDTA, how
a sharp end po
solution and as
point color cha
stable.) A corre
performing a "b
It is more cor
This is preparec
solutions and a
turns a dull viol
this is true, one
EDTA will turn
When Mg-EC
the Mg^{2+} (sinc
indicator. At th
Mg^{2+}, causing
procedure is us
An alternativ
immediately re
.This in effect re
the Mg^{2+} by
adjusted). Whe
titration is start
end point, it ch
correction is re
zation. This sol

High purity l

The waters of
The titratio
ammonia–amr
may precipitat
titrated in the
precipitates ar
Since Ericoc
somewhat on
colors. For exa
and In^{3-} is yel
The pH also aff
ion, as well as
An indicato
stable comple
rather comple
with several d
Calmagite i
the titration o

The chart is interesting. Not only does it show the "minimum pH for detection", I think it is actually showing (indirectly) the detection level itself. Notice our equivalence point for Fe^{+2} was reached @ pH of ~5.07 - right above the min. detection level shown on the chart (i.e. ~4.5 to 5)

We should be very close to the detection limit in our case.

Now, our next question, is Can we identify. Issues are

1. Detection — yes
2. Concentration — presumed to be determinate
3. Identity — A very big question.

To identify I believe we will need to equivalent of the pKa - Ka table for of acids for EDTA - element - dissociations for titrations

From Christian, charts such as the do exist. Now where to find them.

EDTA is a amino carboxylic acid.
EDTA is a weak acid (but pH is going up)

One article is mentioning that buffers are used to maintain a constant pH during a complexation titration. We are certainly not doing this these far.

I am not finding my idea in the literature that it seems like it should work.

It would seem like each ion (but maybe it is each compound
eg $CaCl^{?}$ may each have their own dissociation value.
$Ca(OH)_2$?

I think the issue be the problem.
It does look like no standard methodology for metal ion identification (not concentration) exists via EDTA titrations. Seems like a good idea, similar to how I am identifying an acid.

June 10 2019

Unfortunately, titration (EDTA Complexometric) does not seem to be a method that is used for identification of metals. It still seems like a viable idea to me.

It seems as though I should be able to determine a $K_{Fe^{2+}}$??

In the most recent run (very weak solution) we have an equivalence point @ 1.52 ml and the pH was 4.58; adjusted to 5.18

½ Equivalency point occurs therefore @ 0.76 ml
Our current value is 0.76 ml / .032 ml/count ≈ 24
We do not have the count data available but we can interpolate.

n = 0 pH = 3.09
n = 41 pH = 4.45 (immediately prior to equivalence pt.

Therefore $(24/41)(4.45 - 3.09) = 0.29\ (\Delta pH)$

Therefore our estimated pH @ the half equivalence point is 3.09 + 0.29 = 4.18
And then is adjusted to 1.19(4.18) − 0.27 = 4.70 = $pK_{Fe^{2+}}$
Therefore our estimate for $pK_{Fe^{2+}}$.
and

$$K_{Fe^{2+}} \overset{?}{\approx} 10^{-4.70} = \underline{2.00E\text{-}5}$$

There is a hypothetical proposal for Fe^{2+}, can this be used as a means by identification??

Now let's work up the prior case at moderate concentration and see what we find.

Notice how surprisingly close our unadjusted pH equivalency point are, regardless of concentration.

~~Trial 1 4.395~~
~~Trial 2 4.58 4.395~~
~~Trial 3 4.58~~

Trial 2: 4.395
Trial 3: 4.58

Now let's look @ ½ equivalency point for Trial 2*

Initial pH
n=0 pH = 3.90
n=5.5 pH = 4.395 Vol = 1.77 mL

so half equivalency point occurs @ a volume of 0.885 mL
Count = (0.885 mL / 0.321 mL/count) ≈ 3

@ n=5 pH = 4.15 (immediately prior to equivalency point
Therefore (3/5)(4.15 − 3.90) = 0.15

∴ $pK_{Fe^{2+}}$ = 3.90 + .15 = 4.05 unadjusted

Adjusted = (1.19)(4.05) − 0.27 = 4.55

$K_{Fe^{2+}} = 10^{-4.55} = 2.81E-5$

Hypothetical Method

$pK_{Fe^{2+}}$? $K_{Fe^{2+}}$?

Therefore our two estimates for $pK_{Fe^{2+}}$ & $K_{Fe^{2+}}$ are:

	$pK_{Fe^{2+}}$	$K_{Fe^{2+}}$ $(=10^{-pK_{Fe^{2+}}})$
Trial 2	4.70	2.00E-5
Trial 3	4.55	2.81E-5

X: $pK_{Fe^{2+}} = 4.625$ X: $K_{Fe^{2+}} = 2.40E-5$

Interesting hypothesis development & Computation. Both valid and reasonably consistent even though concentration varies considerably.

I would like to try a random concentration and see if I get similar results.

10.04 gms $FeSO_4$ solution.
25.00 gms total
Initial pH is 4.89. Acidify to 3.95

Vol = 12.3 ml pH = 4.91 n=69] problem here
Vol = 12.0 pH = 5.58 n=39]

You must prime the column.

There was a total ~~blunder~~ burst. Your point was passed without proper detection and the volume flow was inconsistent.

Let's try titration again.

Maybe slow speed was better.

10.0gms $FeSO_4$
~~25.04~~ gms total volume 25.00
Initial pH ~~5.44~~ 4.70 - Acidify to 3.95

You can photograph your data set collection if required

Fast	Volume = 12.0 ml	pH = 5.00	n=41
	Volume = 12.0	pH = 5.55	n=39
	Volume = 12.0	pH = 5.76	n=43

There seems to be a problem occurring here.
Acidify the solution further.

Slow	Initial pH = 4.93	Acidify to 3.54 (~6 drops HCl)	

Column must be primed to get reliable titration results
Titration was really better in slow mode
now the column being primed in fast mode
twice.

Slow	Volume = 3.0 ml	pH = 3.61	n=80	(.0375 ml/count)
Slow	Volume = 3.0 ml	pH = 3.78	n=79	
Slow	Volume = 3.0	pH = 3.93	n=82	
Fast	Volume = 12.0	pH = 4.94	n=40	(0.30 ml/count)

The reference pt pH we going to need to be standardized I do believe.

Our strongest slopes spread between

$n = 15$ pH = 4.31
$n = 22$ pH = 4.67
$\bar{n} = 18.5$ $\bar{pH} = 4.465$

adj pH
adj pH = 1.19(4.465) − 0.27 = 5.04 Estimated eq. pt
with a volume of
3 ml
+ 3 ml
+ 3 ml
+ (18.5) ml (0.30 ml/count) = 14.55 ml

Therefore our ½ equiv pt = 7.725 ml
and count = 7.725 / 0.30 ml = 24
and our pH @ n=24 = 4.69 which is higher than our equiv pt. So we have a problem here.

Notice Trial 2 & 3 both had an end pt pH of 3.89 & 3.90. This is important.

Here is something you see (& you have seen the before). $FeSO_4$ oxidizes in H_2O over time and changes the composition of the solution to the oxidized form. You can never get the same results.

We are now going to look closer at the effects of pH upon the titration results.

The $FeSO_4$ solution is now fresh again. I believe it must be acidified to reduce oxidation. I have added 1 drop 10M HCl to the 200 ml stock solution. We are going to attempt a steady pH of 3.9 to maintain comparison w/ previous trials 2 & 3.

10 ml 0.1M $FeSO_4$
25.3 g add to total volume H_2O ml
Initial pH is 4.69 Acidified to pH = 3.79
Slow Solution is clear.

Volume = 3.0 ml pH = 3.96 n = 83 (.036 ml/n)
Volume = 3.0 pH = 4.32 n = 80
Volume = 3.0 pH = 4.60 n = 83

There is no discernible slope break. Somehow I've changed here? What?

Initial pH = 4.10 Acidify to 3.88
Volume = 12.0 ml pH = 4.96 n = 80 (0.150)

I adjusted the Hoffman clamp. Nevertheless, the flowrate is not @ all constant.

You do not have an equivalence point reached w/ a steep slope. I have no idea why. You don't have. I do not see where I changed.

Pressure in a water column = $\rho g h$

ρ = density (a constant)
g = acceleration due to gravity (a constant)
h = a variable

Therefore $\frac{dP}{dh} = (\rho \cdot g)$ or $\Delta P = (\rho \cdot g)\, dh$

Therefore pressure at top of column or any where along the column is linear. We have a 2-to-1 ratio.
9 ml mark is ~ 22 cm above eye dropper.
-3 ml mark is ~ 22 cm above the 9 ml mark.
So in general: Flow rate @ -3 ml mark = 2x
Flow Rate @ 9 ml mark = x
Flow rate midway $\cong$ 1.5x

So	x(h)	y		x(ml)	y
@	-3	2x	let x = 1	-3	2
@	9	x	2x = 2	9	1

Flow Rate = $-.083$ (height in ml) + 1.75
So 1, 1 1/2, 1 1/2, 1 3/4, 2 drop sizes should be adequate for all work.

Now we notice after the solution has been sitting for a long time the pH has risen to 6.94.

It has also turned a clear golden color. So obviously we had a reaction but it was delayed.

So how do we deal w/ that one? Why is this happening now but did not happen before? a very pretty color now.

The only thing different that I know of now is that the EDTA solution is not fresh.

Let's reduce the volume by 1/2

0.1M EDTA solution = $\frac{18.612 \text{ gms}}{500 \text{ ml}} = \frac{X}{250 \text{ ml}}$

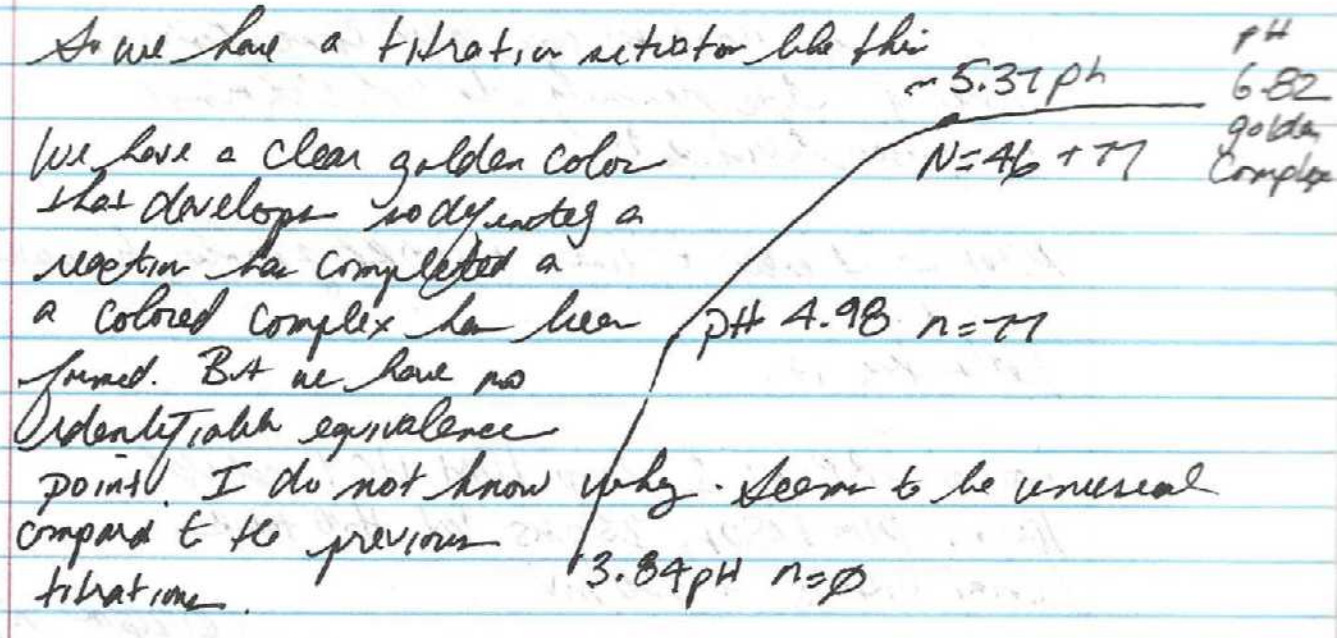
$X = \frac{9.36 \text{ gms}}{\text{in } 200 \text{ ml distilled } H_2O}$

Initial pH 6.23 10gms $FeSO_4$ / 25.00gms H_2O in
Acidify to 3.84

Vol = 12.0 ml pH = 4.94 n = 17 (0.156 ml/n)
Vol = 12.0 ml pH = 5.45 n = 18

pH stabilizes @ 5.37 but there is no steep slope that precedes it.

So we have a titration curve like this



We have a clear golden color that develops indicating a reaction has completed a a colored complex has been formed. But we have no identifiable equivalence points. I do not know why. Seems to be universal compared to the previous titrations.

It seems as though the 4.94 region is as clear as anything at least to a close interval.

If we look @ the mid point of (3.84 + 4.98)/2 = 4.41 pH it is close to the pK#2 determined earlier but there is no justification for it.

So our issue is:

1. We do have a definite reaction that takes place slowly and produces a colored complex.

2. The reaction proceeds gradually with no identifiable equivalence point.

Thus is ultimately, therefore, not a suitable analytical titration.

Let's revert back to low level concentrations of $FeSO_4$. Incidentally the color of mixed is quite beautiful.

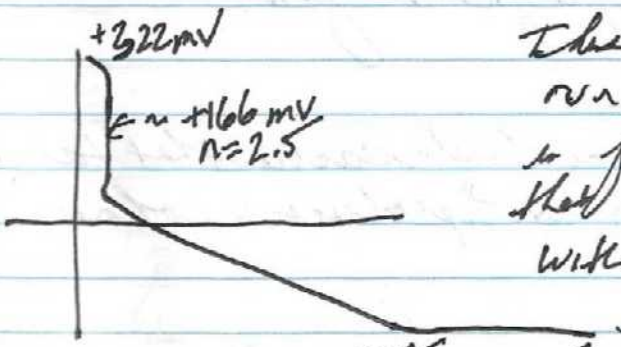
What if I were to use the ORP probe, however?

Let's try it.

Initial ORP w/ 1 drop 10M HCl added
10gms 0.1M $FeSO_4$, 25gms Vol H_2O total
Initial ORP is +320 mV

Volume = 12.0 ml ORP = −130 mV n = 95 (close!) (0.126 ml/n)

So let's see what happened. We had an unusual, rate radical change @ n=2 from +274 mV to +50 mV, a Δ of 216 mV.



The talk is that our EDTA solution is far too strong and that we may be dealing with other ionic species in solution.

Color continues to develop slowly here - gold color

You must use distilled water (and you are) for the EDTA solution, as it will combine and react w/ minerals otherwise.

We are using 0.1M. This appears to be way too strong.

It should be 0.01M, not 0.1M

It should also be used fresh.

Our curve must indicate that we have more than one ion in solution. We know that we have an a mixture, Fe^{2+} & SO_4^{2-}.

You can titrate in a stepwise fashion, eg
Fe^{3+}, Al^{3+}, Mn^{2+}, (Ca^{2+}, Mg^{2+})

June 24 2019

Scheduled departure for the northern Coventry is on July 5, + de -.

We see from the attached paper that pH appears to be a tool that can indeed be used for metal identification, not dissociation as was surmised (at least not yet).

I am sorry to look @ the use of the

1. ORP meter
2. a radically more dilute EDTA solution, of 0.01M EDTA vs 0.1M.

Current volume of remaining 0.1M EDTA is 97.67 mL

Lets dilute the to a 0.01 M solution

0.1M → 0.01M : 9(97.67ml) = 879.03ml to be added.

Use 0.27 mole gram FeSO4 in 25.13 g/ml H_2O

879.03
−490.96
= 388.07
−254.44
= 133.63

The ease of actually accomplishing this will depend upon the concentration of each ion in solution. Big differences means big problems!

Example of the use of pH w/ EDTA - Identification is possible.

Continuous determination of iron, aluminum, manganese, calcium and magnesium mixture by EDTA titration

Abstract

An acidic solution of hydrochloric acid or perchloric acid cotaining Fe^{3+}, Al^{3+}, Mn^{2+}, Ca^{2+} and Mg^{2+} is adjusted to pH 2.0 by addition of ammonium acetate and the Fe is titrated with a standard EDTA solution using salicylic acid as an indicator.

The solution after determination of Fe is treated with EDTA in a slightly excess for Al, adjusted to pH 3.0, heated to boil for the reaction Al and EDTA and the excess of EDTA is titrated with a standard copper sulfate solution using PAN (α-pyridyl-β-azonaphthol) as an indicator.

The above procedure is the same as in the usual method. The solution after determination of Al is adjusted to pH 4.5, heated up to 60°C, EDTA is added in a slight excess for Mn, kept for 10 min and the excess of EDTA is titrated with the standard copper sulfate solution with PAN indicator for determination of Mn without the influence of Ca^{2+} and Mg^{2+}. Although the formation constant of Mn chelate is found to be greater than that of Cu chelate, Mn^{2+} showed a red coloration with PAN and the end point of titration can be found without difficulty.

Based on the fact of determination of Mn from a solution of pH 4.5, a continuous determination of Fe, Mn, Ca by titration with EDTA becomes possible. Namely, the solution after determination of Mn is used for determination of Ca and Mg in alkaline medium as usual and the procedure is carried out as follows:

A part of solution after determination of Mn is taken, adjusted to pH 10, EDTA is added in a slight excess for total sum of Ca and Mg, the excess of EDTA is titrated with the standard copper sulfate solution and the sum of Ca and Mg is calculated. The other part of solution is treated with ammonium oxalate solution for precipitation and separation of Ca, and the solution is adjusted to pH 10 and the Mg is determined in the same way as in the detemination of the sum of Ca and Mg.

In case of dealing with a solution of Al-EDTA alone of pH 10, it caused the precipitation of aluminum hydroxide with the liberation of EDTA and the determination became impossible, but it was found that aluminum hydroxide remained unprecipitated in the presence of Fe-EDTA and Mn-EDTA and there was no disturbing influence for titration (Ca+ Mg) and Mg.

Initial ORP: +413mV
You forgot to acidify the solution.

0.34 gms $FeSO_4$ in 25.03 ml total H_2O

ORP initial = +407 (pH* (ORP) meter reads 6.83)
add 2 drops 10M HCl
The drop the pH (as read by the ORP meter) to be very similar to actually using the pH meter. The shows ORP & pH meter (probes) to be acting in a similar fashion.

Acidified solution: pH* = 3.53 / Initial ORP = +359mV
Now we slow mode.

n=92
Slow Volume = 3.0 ml mV = +105 and increasing slowly
It actually reached a maximum @ n=79

Flow Rate = 3.0/92 = 0.033 ml/count.

So here we have a fairly steady decline until a minor minimum is reached @ n=79 (+89mV)
Very interesting.
79 (.033) = 2.58 ml

The solution remains clear pH* measures 6.06
mV has now increased to +137mV
This equivalent to a backtrack of n=29.

One unexpected change in ORP occurs, once again, in the first 2-3 drops.

At the minimum reached here that is interesting. Now continuing to increase @ +151 mV ($n = 24$)
It does appear that there is color in the solution. This suggests the reaction actually is complete.

We have noticed quite beautiful colors continue to develop as the analyte rises; ie, orange, red, yellow, etc.

In the pH* mode we see that the pH* did cross some kind of equivalence point, reached max ~ pH* ≅ 7.0, and is now diverging back to an acidic state, currently @ 5.62

Question: Is the ORP minimum reached at the maximum pH* reached, or @ the point of greatest change in pH*?

ORP now @ +180 mV ($n \cong 16$)

Let's repeat the test.

Our solution is transparent w/ a slight yellow-green color identical to the manner of the early titrations. Maybe you should be dealing w/ low concentrations only.

Use 0.5 gm $FeSO_4$ in 25.00 total ml H_2O
Solution appear perfectly clear @
the concentration level.

Initial ORP = +219 mV ($pH^* = 5.08$)
Acidify to ORP = +
ORP
Let's use pH^* scale the terms (mV) again

Initial mV = +350 ($pH^* = 3.61$) 3 ml/100(3) = .0300 ml/count

Volume = 3.0 ml mV = +82 $n \Rightarrow 100+1$
But now it is rising again, so you ran over.
You must increase the flow rate some.
mV now @ +106

So we have exactly the same behavior.

$M_1V_1 = M_2V_2$

For first case

$(.01\,M)(2.58\,ml) = (25.03\,ml)M_2 \Rightarrow M_2 = .0010$

Second Case: 100(.0300 ml/count) = 3 ml

$(.01M)(3.00\,ml) = 25.00\,ml \cdot M_2$ $M_2 = .0012$

Now, in our first case, solution strength =

$\frac{0.34\text{gms}}{25.03\text{ml } H_2O} = .0136$ or 1.36%

in second case

$\frac{0.57}{25.00} = .0228$ or 2.28%

$\frac{2.28\%}{1.36\%} = 1.68$ ratio & $\frac{.0012}{.0010} = 1.20$ ratio.

Hard to say here, however the thickness of the solution is highly concentrated.

We also have exactly the same Cola formation. Let's use the pH meter again.

Use 0.33gms $FeSO_4$ in 25.03 total ml H_2O
Initial pH = 5.83, Clear solution, acidify to 3.64
Increase flow rate slightly.

Slow Volume = 3.0 ml pH = 3.67 n = 69
We are measuring two different reactions taking place. No sharp change detected.
Change to FAST mode

FAST Volume = 11.5 ml pH = 4.45 n = 23 (0.50 ml Count)
flow rate is TOO FAST slow it down

Volume = 0.2 ml pH = 4.45 n = 5
(TOO Slow)

Volume = 12.0 ml pH = 4.95 n = 82
No steep curve

Volume = 12.0 ml pH = 5.10 n = 34
5.0 ml pH = 5.14 n = 47

No specific Point of key reaction here. The high titration method should be used. ORP appears repeatable and sensible in terms of color development.

Use 3.08 gms $FeSO_4$ in 25.05 total ml H_2O.

Use ORP meter.
Initial ORP = +316 mV. Acidify to +365 mV

Fast

Volume = 12.0 ml ORP = -49 mV n = 43
Volume = 12.0 ml ORP = -82 n = 44

I seem to have lost sensitivity here.
No minimum is reached.

There seems to be some unusual behavior here.

One apparently successful trial used:
1. 2 ml of $FeSO_4$ in 200 ml total H_2O
3. 5 drops of $FeSO_4$ in 200 ml total H_2O.

Slope change occurred in both cases near pH 4.5 after acidification to pH ~ 3.9 - 4.0.
titration

Notice volumes used in both cases are similar so there is a problem. Also note that 0.1 M EDTA was used & this may be a/o the problem.

Let's look @ use of pH again.
Lets start w/ low concentration and work up from there.

Question: If the original pH is a variable, does the product/variable results?

This column became crossed in formation. Recorded it.

1 drop $FeSO_4$ (0.5M) in 25 ml H_2O
Initial pH = 6.81 Acidify to 3.72 pH - 2 drops HCl

				ΔpH
Slow	Vol = 2.50 ml	pH = 3.77	n = 83	Δ = .05
	Vol = 2.50 ml	pH = 3.91	n = 83	Δ = .14
	Vol = 2.50 ml	pH = 4.10	n = 83	Δ = .19
	Vol = 2.50 ml	pH = 4.31	n = 81	Δ = .21 Σ = 10ml
	Vol = 2.50 ml	pH = 4.55	n = 81	Δ = .24 ← MAX
	Vol = 2.50 ml	pH = 4.73	n = 78	Δ = .18 Σ = 15ml
	Vol = 2.50 ml	pH = 4.84	n = 80	Δ = .11

The was a very revealing trial. We have now reclaimed our repeatability. The slow mode has revealed the sensitivity and detection limits of the method; we may have bypassed them previously w/ the use of the fast mode.

Clearly we do have a maximum @ pH ≅ 4.55 but it is not detectable at the individual count level

Our adjusted pH here = 1.19(4.55) − 0.27 = 5.14
@ Volume ≅ 12.5 ml. Our ½ equiv. pt is at Volume ≅ 6.25 ml which corresponds to a pH of ≅ ~~4.00~~ 4.01 and the adjusted ½ equiv pt = 1.19(4.01) − .27 = 4.50 = $pK_{Fe^{+2}}$ and this leads to a $K_{Fe^{+2}}$ of ≅ $10^{-4.50}$ = 3.2E-5

We now have three estimates for $pK_{Fe^{2+}}$ & $K_{Fe^{2+}}$

Equiv Point

$pK_{Fe^{2+}}$		$K_{Fe^{2+}}$
4.70		2.00 E-5
4.55		2.81 E-5
5.14	Actual Value on EDTA chart ≈ 5.1 good	3.2 E-5
$\bar{x}$ = 4.8		$\bar{x}$ = 2.7 E-5

We see now that concentration levels & appropriate titration rates are crucial to the detection and the results.

Your hypothesis of a $pK_{Fe^{2+}}$ and $K_{Fe^{2+}}$ looks to be perfectly valid and eventually very useful.

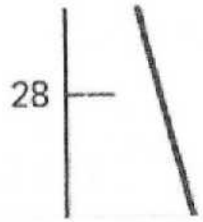
I do absolutely think that the method can be used for identification of species. Most metals that complex w/ EDTA will do so @ a unique pH and they're a unique K_{Metal}

The is fantastic and it was the original hypothesis to begin with.

We see that we are right on range. EDTA shows pH detection @ ~ 5.1 for Fe^{+2} vs our 4.8. The is good work.

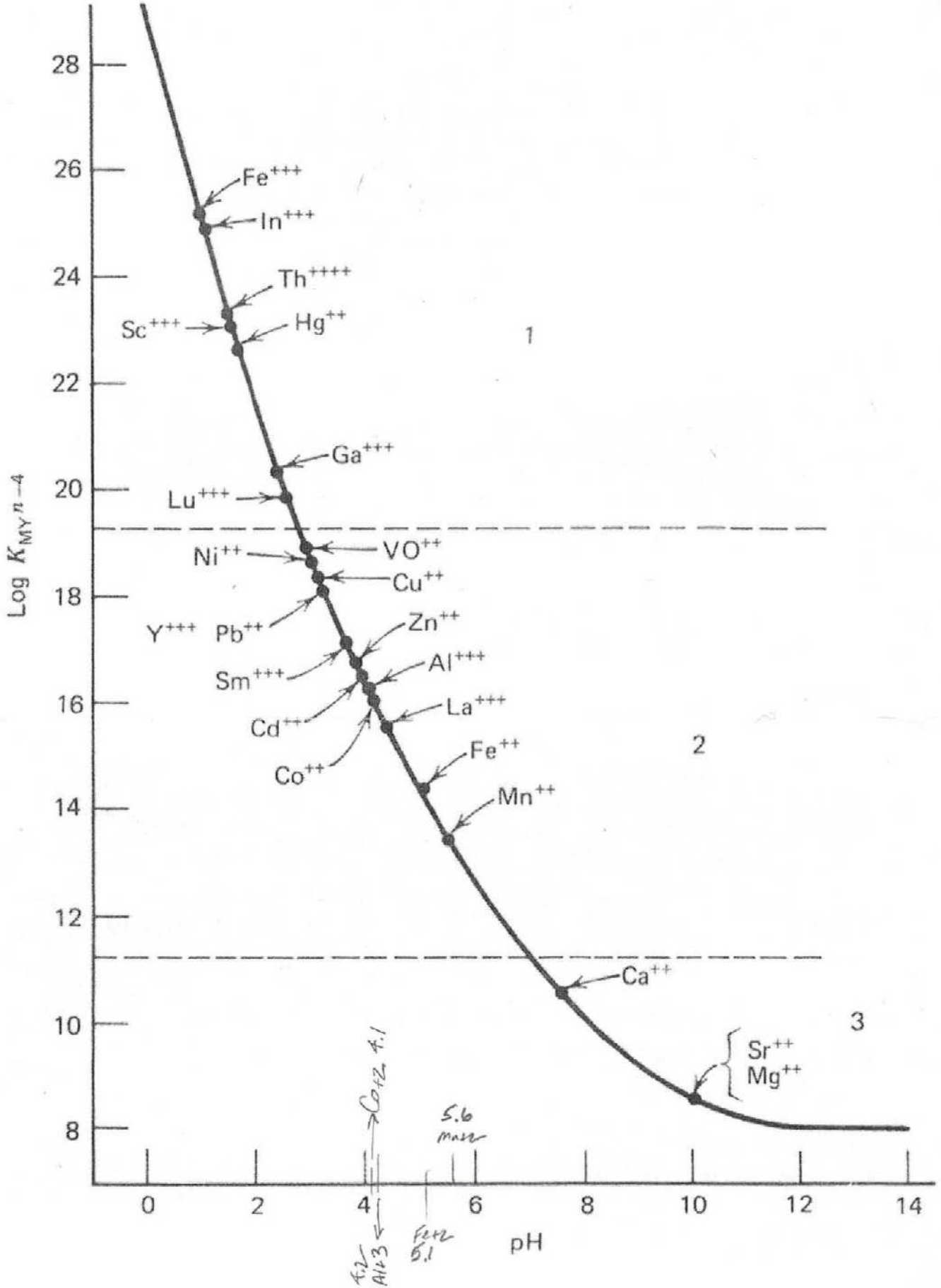
Incidentally, our latest determination is right on track.

EDTA Minimum pH Chart for Metal Ions



Enlargement of EDTA-pH Minimum Detection Chart

EDTA Minimum pH Chart for Metal Ions



1 drop $FeSO_4$ (0.5M) in 25.16 mL total H_2O

1 drop $\approx$ .05 mL, $\frac{25.16\text{ mL}}{.05\text{ mL}} = 503$

so our solution concentration should be $\approx \frac{0.5M}{503}$

$= .0010\ M\ FeSO_4$

It looks like this is exactly what we computed during a couple of our titration examples. We will see? Recall these were based upon ORP measurements.

Recall that we have moderately acidified our 0.5M stock $FeSO_4$ solution to reduce oxidation over time during storage.

Let's use the fast mode this time.

Initial pH = 5.90 / add 3 drops HCl. Acidify to pH 3.66

Fast — Volume = 12.0 mL pH = 4.23 n = 43 $\Delta pH/n = 1.3E{-}2$

Switch to Slow Mode.

Look @ data closely.

Slow — Volume = 2.50 mL pH = 4.43 n = 80 $\Delta pH = .20$

n_1	n_2	pH_1	pH_2	ΔpH	$\Delta pH/n$
1	20	4.24	4.29	.05	2.5E-3
21	40	4.30	4.34	.04	2.0E-3
41	60	4.34	4.39	.05	2.5E-3
61	80	4.39	4.43	.05	2.5E-3

The lesson here is that it is not always suitable to use $\Delta n = 1$ to make slope determinations. It depends upon concentrations of both titrant & analyte.

Slow: pH = 4.60 Vol = 2.50 ml n = 84

n_1	n_2	pH_1	pH_2	ΔpH	$\Delta pH/n$
1	20	4.41	4.45	.04	2E-3
21	40	4.46	4.50	.04	2E-3
41	60	4.50	4.54	.04	2E-3
60 61	80 84	4.54	4.59	.05	2.1E-3

We see now that $\Delta pH/\Delta n$ is a critical value and that it should always be recorded at least at some level.
Weaker solutions require a larger Δn interval to detect a change. If you can not detect a change when you expect one, slow down the titration and increase the $\Delta pH/\Delta n$ interval.

Vol = 2.50 ml pH = 4.71 n = 84 $\Delta pH = \pm 0.11$
We therefore see that the max $\Delta pH/\Delta n$ was reached @ pH ≅ 4.4 Equivalence Point
Our adjusted pH is therefore $(1.19)(4.4) - 0.27 \cong 5.0$
The volume occurs @ ~~12.5~~ 14.5 ml. ½ volume = ~~6.25~~ 7.25 ml
We have only an approximate estimate for pH at
~~Flow Rate ≅ 12/43 = 2.8E-1 ml/ml~~
~~6.25 ml~~ $[(6.25/12\ ml)(4.23 - 3.66)] + 3.66 \cong 4.0 = pK_{Fe}$
or
~~$K_{Fe^{2+}} = 10^{-4} = 1E-4$~~
Adjusted ½ Equiv Point = $1.19(4.0) - .27 = 4.5 = pK_{Fe^{2+}}$
or $K_{Fe^{2+}} = 10^{-4.5} = 3.2E-5$

We now have 4 estimates for $pK_{Fe^{2+}}$ & $K_{Fe^{2+}}$

Adjusted

~~Equivalence Point~~ ~~pKFe2+~~	$pK_{Fe^{2+}}$ adj.	$K_{Fe^{2+}}$
~~4.70~~		2.00E-5
~~4.55~~		2.81E-5
5.14	4.5	3.2E-5
5.0	4.5	3.2E-5

$\bar{x} =$ ~~4.8~~ $\bar{x} =$ ~~2.8E-5~~

Final Values

Notice, however that our last two trials were completed under much tighter control w/ a high level of repeatability.

Notice also we take the average of these two last headings we end up with an average value right of Equiv Pt (Theoretical 5.1) also

~~$pK_{Fe^{2+}} \cong 5.1$~~ $\frac{1}{2}$ Equiv Pt $pH \cong 4.5 = pK_{Fe^{2+}}$

$K_{Fe^{2+}} = 10^{-4.5} \cong 3.2E-5$

Final value. Thus good work.

X You can indeed very likely identify a metal ion species by titration

We can also determine the concentration of Fe^{2+}.

EDTA $FeSO_4$

$M_1V_1 = M_2V_2$

$.01(14.5\,ml) = (25.16\,ml + 0.15\,ml\ HCl) \cdot M_2$

$M_2 = 5.7E\text{-}3M = .0057\,M$ molar concentration of $FeSO_4$.

I anticipate that you may be able to detect $\pm .001M$. This is equivalent to roughly 1 drop of $0.5M$ $FeSO_4$ in ~ 1 ml of H_2O vs 25 ml of H_2O.

You must use distilled water for the process.

June 25 2019

Let's go for extreme sensitivity testing w/ no expectations of success.

Use 1 drop $FeSO_4$ (0.5M) in 1000 ml. $FeSO_4 \cdot 7H_2O$
The is equivalent to approx

$$.5M = 0.5\left(\frac{278.02 gms}{1000 ml}\right) = \frac{139.01 gms}{1000 ml}$$

In one drop we have approx

$$.05 ml \left(\frac{139.01 gms}{1000 ml}\right) \tilde{=} .007 gms$$

So we take

$$\frac{\frac{.007 gms}{1000 ml}}{\frac{278.02 gms}{1000 ml}} = \frac{.007 gms}{278.02 gms} = 2.5E\text{-}5M$$

There is no way we should be able to detect this concentration level. But for kicks, let's see.

This was unrealistic. I have added 10 drops instead. This will bring concentration to

2.5E-4M still theoretically out of range by one order.

Use 25.00 ml of this 2.5E-4M solution.
Initial pH = 6.78
Add 3 drops HCl ⇒ 25.15 ml total volume
Acidify, therefore, to pH 3.62. Use slow mode

FRAC
Slow Volume = 2.50 ml pH = 3.70 n = 88 2.8 E-2 ΔpH/n = 9.1E-4
Fast Volume = 6.90 ml pH = 4.08 n = 23 3.9 E-1 ΔpH/n = 1.7E-2
Flow rate of fast is 11 times greater than slow.
Adjusted equivalent flow rates, we have 1.7E-2/11 = 1.5E-3 ml/n
vs 9.1E-4
Back to slow speed

SLOW Volume = 2.5 ml pH = 4.26 n = 87

n_1	n_2	pH_1	pH_2	ΔpH	ΔpH/n
1	20	4.09	4.13	.04	2E-3
21	40	4.13	4.17	.04	2E-3
41	60	4.17	4.21	.04	2E-3
61	80	4.21	4.25	.04	2E-3
80	87	4.25	4.26	.01	1.4E-3

Volume = 2.5 ml pH = 4.44 n = 86

n_1	n_2	pH_1	pH_2	ΔpH	ΔpH/n	
1	20	4.26	4.29	.03	1.5E-3	
21	40	4.29	4.34	.05	2.5E-3	Max
41	60	4.35	4.38	.03	1.5E-3	
61	86	4.38	4.44	.04	1.6E-3	

Volume = 2.5 ml pH = 4.62 n = 86

n_1	n_2	pH_1	pH_2	ΔpH	ΔpH/n	
1	20	4.44	4.48	0.04	2E-3	
21	40	4.48	4.53	0.05	2.5E-3	Max
41	-60	4.53	4.56	0.03	1.5E-3	
61 -	86	4.57	4.61	.04	1.6E-3	

Vol = 2.5ml pH = 4.74 n = 86

n_1	n_2	pH_1	pH_2	ΔpH	$\Delta pH/n$
1	20	4.61	4.65	.04	2E-3
21	40	4.65	4.68	.04	2E-3
41	60	4.68	4.71	.03	1.5E-3
61	86	4.71	4.74	.03	1.2E-3

So we do have maximum paired values occurring between pH 4.44 & 4.53

Furthermore, our max value study (latest & paired) occurs @ pH = 4.51

There is our equivalence point that has been successfully determined.

Adj. pH equiv pt = 1.19(4.51) − .27 = 5.1

We know theoretical point is 5.1 so we are, once again spot on even w/ a highly dilute solution w/ a concentration of 2.5E-4 M

This exceeds stated expectations in the literature. Marvelous work here.

There actually is a slight amount of color visible in the 1 L. liter stock solution, but none visible in the 25 ml analyte sample

Since color is variable, it makes me wonder, can I detect a single drop?

Let's extract 100 ml and dilute it by a total factor of 10. Done. Sample Size 25.04 ml total. Initial pH is 6.54 Acidify to 3.62
Total Volume now is 25.04 + 0.15 ml = 25.19 ml
Use fast mode for first run:

Fast Vol = 12 ml. pH = 4.21 n = 46
Slow Vol = 2.5 ml pH = 4.43 n = 84

n_1	n_2	pH_1	pH_2	ΔpH	$\Delta pH/n$
1	20	4.23	4.28	.05	2.5E-3
21	40	4.29	4.34	.05	2.5E-3
41	60	4.34	4.38	.04	2.0E-3
61	84	4.38	4.43	.05	2.2E-3

I blew it and used fast mode.
Vol = 10.8 ml pH = 4.94 n = 39

n_1	n_2	pH_1	pH_2	ΔpH	$\Delta pH/n$
1	5	4.43	4.52	.09	
6	10	4.54	4.64	.10	Max
11	15	4.66	4.73	.07	
16	20	4.74	4.79	.05	
21	25	4.81	4.85	.04	
26	30	4.86	4.89	.03	
31	35	4.90	4.92	.02	
36	39	4.93	4.94	.01	

Notice Interval selector of $\Delta n = 5$

We salvaged the data. We detect the max @ pH = 4.59
Adjusted pH = (1.19)(4.59) − 0.27 = 5.2 Equivalence Pt.

So indeed we were able to detect the equivalence point even in the fast mode w/ a judicious selection of $\Delta pH = 5$.

This is really quite amazing as we have now consistently detected the equivalence point for Fe^{2+} this time @ a rather amazingly low concentration, now on the order of

2.5E-5M! by titration. Literature states expectations @ 1E-3M

This equates to a single drop of 0.5M $FeSO_4$ in a liter of water.

I have also run a colorimetric detection test w/ Phenanthroline. The color (red) from the reagent is decidedly not detectable by eye. We might be able to pick it up on the VIS spectrometer, we shall see.

A stronger concentration (ie ~ 100 ul of 0.5M $FeSO_4$ in 1½ ml of water) is detectable by eye.

It is also especially amazing that the detection was made in the fast titration mode, close to an overnight.

Very good inorganic detection (& concentration) methods development has taken place here.

Our last 4 titrations lead us to an Equivalence point determination of

1. 5.1 pH across a variety of concentrations.
2. 5.1 pH
3. 5.1 pH Each done w/ a Complexometric
4. 5.2 pH formation with EDTA.

Your next two questions consider:

1. A mix of Mn & Fe^{+2}.
   Can you detect and separate (ie, identify) these?

2. What about non-aqueous titrations? Not everything is in water. What if, for example, you had an extraction from a HEPA filter into methanol?

These are very realistic and important questions.

Ok, very good news. I was able to pick up the Phenanthroline Fe^{+2} reagent red color test w/ VIS spectroscopy. Absorbance is low but very detectable (~ 0.04) @ a max λ of 510nm. The corresponds to red-(red-purple). I could also detect this by eye looking long ways through the cuvette.

There is a very good secondary test method, therefore

, Up to 3 detection methods: 1. titration 2. Reagent test 3. Electrochemistry.

June 26 2019

Manganese sulfate exists as a monohydrate form. The molecular weight of the is 169.02 gms/mol.
0.1M = 16.9 gms.
Lets use 0.01M Solution.
1.69 gms in 1000 ml.

We have 0.5M solution of $FeSO_4$
Dilution ratio to 0.01M is $\frac{.500}{.01} = 50$
so 1 ml + 49 ml H_2O = to 1 50 ml
10 ml + 490 ml H_2O = 500 ml
Lets use these.

I have created a 25 ml analyte solution:
50% 0.01M $FeSO_4$
50% 0.0 M $MnSO_4$

The goal is to see if the pH Δ between pH 5.1 & 5.6 can be discerned via EDTA Complexometric titration.

Initial pH is 6.47. Add 3 drops 10M HCl Acidify to pH 3.63. Fast mode just.

FAST Vol = 12 ml pH = 4.04 n=47

n_1	n_2	pH_1	pH_2	ΔpH	ΔpH/n
1	2 10	3.62	3.69	.01	.007
2 11	4 20	3.70	3.79	.09	.009
4 21	6 30	3.80	3.89	.09	.009
6 31	40	3.90	3.98	.00	.008
41	47	3.98	4.02	.04	.006

FAST Vol = 12.0 ml pH = 4.63 n = 47

n_1	n_2	pH_1	pH_2	ΔpH	$\Delta pH/n$
4	5	4.07	4.11	.04	.008
6	10	4.12	4.17	.05	.01
11	15	4.19	4.23	.04	.008
16	20	4.24	4.29	.05	.01
21	25	4.30	4.35	.05	.01
26	30	4.36	4.41	.05	.01
31	35	4.42	4.47	.05	.01
36	40	4.48	4.53	.05	.01 ← Max
41	47	4.54	4.60	.06	.0086

Vol = 12.0 ml pH = 4.98 n = 47

n_1	n_2	pH_1	pH_2	ΔpH	$\Delta pH/n$	
1	8	4.64	4.73	~~.07~~ .09	~~.0088~~ .011	
9	16	4.74	4.82	.08	.01	Max
17	24	4.83	4.88	.05	.006	
25	32	4.88	4.92	.04	.005	
33	40	4.93	4.95	.02	.0025	
41	47	4.96	4.98	.02	.0029	

We have, once again identified the max $\Delta pH = 4.505$ or the adjusted pH as $1.19(4.505) - .27 = \underline{\underline{5.1}}$ Spot on again

You have seen that by appropriate selection of intervals that the FAST mode can be used further

Now your question arises, What about Mn^{+2}?

The first question is, what is the standardized pH of the EDTA 0.01 M solution?
6.70
Let's see. It is ~~6.67~~ Unadjusted.
adjusted = 1.19 (6.67) − .27 ≈ 7.7
6.70

This is good. Theoretically we should be able to cross the 5.6 level.
(Unadjusted value ≈ 4.9 pH). Let's try,

Notice our pH has already crossed the point.
Our current pH is ~~5.07~~ 4.99 4.97
If we look at our data set, we can see that we may well have already crossed the Mn^{+2} detection pH ≈ 4.78
The leads to an adjusted value of 1.19(4.70) − .27 = 5.4
vs theoretical 5.6

This is pretty tight quarters but the data seems to reflect that we have already crossed the point. We are quite likely within the experimental error range here. Let's Continue another run:

Vol = 12.0 ml pH = 5.09 n = 47

We definitely have crossed over. ΔpH total = 0.12

ΔpH/Count = .12/47 = 2.6E-3
To equate to last run: (2.6E-3) 8 = .002

It did nothing but slowdown titrate.

The says that we did pick up the Mn+2 but it is soluble, over fast, and a bit tricky to separate out. But I do think that we have done so.

Now repeat the trial again.
I have created a small spreadsheet in my calculator that will speed up the computation process a bit. A fancier titrator would, of course, have a graphical-screen interface but we do not have that luxury. We do, however, have a lot of manual control over a semi-automatic process.

Let's see how the spreadsheet works out.
Initial pH = 6.32 Volume = 24.97 ml (50% each $FeSO_4$ & $MnSO_4$)
Acidify to 3.69 w/ 3 drops HCl → Volume = 25.12 ml
Use Fast Mode
FAST Vol = 12.0 ml pH = 4.03 n = 46
Then great, I have all my values and computations in the Calculator spreadsheet and recording will no longer be needed in the same way. Keep track of volumes.
FAST pH is now registered to 3.99. pH is @ 4.045
Volume = 12.0 ml pH = 4.45 n = 46
We have reached a max holding value @ n = 46 @ 4.45 pH
The spreadsheet is definitely for the automation of the process.
Continue: I can now revisit the entire data history.
FAST Vol = 12.0 ml pH = 4.87 n = 47
4.49 5.1
Max reached @ pH = ~~4.44~~ Unadjusted Adjusted = ~~5.0~~

No second max reached @ this time. Continue.

Vol = 12.0 ml pH = 5.04 n = 47

We do pick up another local maximum @ pH = 4.85 Unadjusted.

It is slight but discernible.

Adjusted pH = 1.19(4.85) − .27 = 5.5

vs theoretical 5.6 pH for Mn^{+2}.

We have indeed done it!
In the second time now, both Fe^{+2} and Mn^{+2} have been detected and identified w/in a mixed solution.

Our concentration levels for both ions is $\frac{.01M}{2} = .005M$ which is not bad at all. The limit of detection can continue to be tested, we know that we have made it to 10^{-5} w/ Fe^{+2}.

We also notice the ΔpH/n for Fe^{+2} is stronger than that for Mn^{2+}.

	Max ΔpH/n:
Fe^{2+}	+.014
Mn^{2+}	+.005

This is very difficult to discern and detect.

You made one mistake in your selection of the n interval. N=46 to 47
You chose Δn to be 7 instead of 8.

This caused you to have a wide interval for the last step ($\Delta n = 11$ instead of 7) and you lost some resolution of the data.

You should have gone $\Delta n = 8$ (ie, 6 intervals * 8 Counts per interval = 48 and your resolution would have been more equally spaced.

It is a great improvement to have the spreadsheet. You can save the entire history of the titration and analyze the data from a broader perspective.

I regard the process as a complete success.

We could now extend and try for three metals. I would suggest we consider adding Ca into the mix.

Before this, however, I think we need to examine the behavior of MnO_2 alone.

Use 25.00 ml of 0.01M $MnSO_4$ Initial pH = 7.63
Acidify to pH = 3.63 w/ 5 drops HCl = 0.25 ml

FAST Vol = 12 ml pH = 3.89 n = 46 Δn = 6 (ΔB)
Estimated max @ ~ pH 4.9 unadjusted

FAST Vol = 12 ml pH = 4.16 n = 47 Δn = 8
We are seeing a small local maximum @ pH ≅ 3.97
Adjusted value = (1.19)(3.97) − 0.27 ≅ 4.5

Yes really do not anything expected here??
Continue

dpH/dN = +.006

FAST Vol = 12.0 ml pH = 4.48 n = 48 Δn = 8
Continue

FAST Vol = 12.0 ml pH = 4.82 n = 47 Δn = 8

We see another local max @ pH = 4.57 (unadjusted)
Adjusted = (1.19)(4.57) − 0.27 = pH of 5.2
dpH/dN = +.009

The suggests some Fe+2 contamination?
Continue:

FAST Vol = 12.0 ml pH = 4.97 n = 47 Δn = 8

Local max @ pH ≅ 4.80 dpH/dN = +.006

Adjusted = 1.19(4.80) − 0.27 = 5.4

Source of Fe+2 suspected to be the Tap Water!

We have unadjusted local maximums taky place at:

	dpH/dN	Unadj ~pH	Adj pH	Candidate Species	Theoretical pH
Line 35	+.006	4.79 4.81	5.5	Mn+2	5.6
Line 30	+.008	4.47	5.0	Fe+2	5.1

Both Fe+2 & Mn+2 show detection. This suggests we have Fe+2 contamination in our analytes. This can be from the original powder reagents or possible from glassware though the sequence unlikely.

Again we see that Mn+2 appears to be more difficult to detect than Fe+2.

Also, since we used a titrant volume of 60 ml vs the titrant 25 ml, we do not need to acidify the solution nearly as much as we did. Two drops vs 5 would have been fine.

I am generally quite impressed with the method and its sensitivity. I do have question as to why Fe+2 seems to be obviously existing within what should be an exclusive Mn+2 sample.

Next we might consider Oxygen Ca into the picture.

Nile: We used TAP water for the Mn Solution. There may well be the source of the Fe+2!

June 21 2019

There is a significant question that has arisen here. How does a sample that is understood to contain $MnSO_4$ show such a strong signal with Fe^{2+}.

Even understanding that tap water was used the presence of Fe^{2+} remains questionable.

Now prepare a distilled water sample of $MnSO_4$.

313.42 ml H_2O distilled
0.17 gms $MnSO_4 \cdot H_2O$
313.59 gms total

$MnSO_4 \cdot H_2O$ MW = 169.02 gms/mol

$\frac{0.17 \text{ gms}}{169.02 \text{ gms}} \Rightarrow .0010M$ solution in 1000 ml

But we have 313.42 ml so $.0010M \left(\frac{1000 \text{ ml}}{313.42 \text{ ml}}\right) =$

$= .0032M$ solution of $MnSO_4 \cdot H_2O$
Then we have to work backwards.

Analyte Sample = 25.16 ml $.0032M$ $MnSO_4 \cdot H_2O$

Initial pH 7.07 Acidify to pH 3.85 (2 drops only HCl)

Vol = 12.0 ml pH = 4.45 n = 45 Δn = 8
Vol = 12.0 ml pH = 5.09 n = 48 Δn = 8

We have a very strong peak @ ~ pH 4.62 dpH/dn = 0.021

Adjusted = (1.19)(4.62) − .27 = 5.2

And there is still close to Fe+2 than Mn+2. But the time we have a very strong signal and we know that we are dealing w/ Mn+2. There also do not appear to be multiple signals there for.

At most you might interpret a pH @ 4.71
The leads to adjusted pH of 1.19(4.71) − .27 = 5.3
Where at first is midway between Fe+2 & Mn+2.
I will complete an additional run however @ the time it is difficult to say whether or not we can successfully differentiate Mn+2 from Fe+2.
We do, however, have very strong signal detection w/ no apparent competition and therefore know that we are dealing w/ Mn+2 here.

We do therefore have a very successful titration take place. We also have the titrant - analyte concentration balanced.

Volume = 12.0 ml pH = 5.24 n = 48
No additional peak

The was a very successful titration w/ a regular decrease (maximum reached @ pH on 4.6 to 4.7

Adjusted values are therefore pH 5.2 to 5.3

The is midway between the Fe+2 & Mn+2 pH points.

∴ We are to accept the difference as being w/in experimental error as we know we have a clean Mn^{+2} sample in the case.

Distilled water most certainly made a difference here in removing competing or conducting signals, however seldom will we have such opportunities w/ real world or sample such as rainwater.

Now let's try a calcium addition w/ a skew to a high pH along the way.

I have a previously made solution of $Ca(OH)_2$ (made from slaked lime and NaOH).

It should suffice fine. It is of unknown concentration.

Let's mix Mn^{+2} & $Ca(OH)_2$

2.04 ml $Ca(OH)_2$ solution
2.04 + X = 13.54 grams w/ $MnSO_4$
Added H_2O to 24.97 grams total volume.
Initial pH = 7.96
Ca pH detection ≅ 7.5 (~6.5 unadjusted)

I forgot to acidify the solution. The good news is that EDTA was able to push the pH to 8.11. It should be no problem to reach the Ca equivpt. Let's try to recover by acidifying the solution.

5 drops HCl have reduced the pH to 3.94

Initial pH = 3.94
Vol = 12.0 ml pH = 4.39 n=48
Vol = 12.0 pH = 4.03 n=47

Our first max is reached @ pH = 4.6 (adj = 5.2)
The compound tells you that you cannot really titrate with FeF_2 & MnF_2. You need a further test.
pH Vol = 12.0 pH = 5.00 n=47
Vol = 12.0 pH = 5.09

I have added considerable 1M NaOH to bring the pH up to 6.09. The solution is acting as a strong buffer.
Vol = 12.0 pH = 6.14 Still acting as a buffer.
Vol = 12.0 pH = 6.18

Ok here's what happened. Presumably the reaction was going to take too long w/ EDTA I added 1M KOH by eyedropper to approach a pH of ~ 6.5.

The solution then began acting as a very capable buffer.

The equivalence pt was reached quite suddenly in the pH range of ~ 6.5 to 7.0, and therefore the Ca detection approach is working perfectly.

You will do exactly as you did, carefully control the pH close to the point of equivalence, then switch to EDTA titration once again.

—

The next trial we will use $Ca(OH)_2$ by itself w/ no acidification.

24.97 ml total volume, $Ca(OH)_2$ only. 10^{-2}

Initial pH = 8.52

Acidify carefully to pH = 5.78

Vol = 12 ml pH = 6.15
Vol = 12 ml pH = 6.27
Vol = 12 pH = 6.35

Max pH reached = 6.86; no definite crossover.

I have learned some lessons here.
1. pH control will be important
2. Volume of burette can be adjusted (now 60 ml instead of 12) to accomodate uncertainty in results.
3. Large burette will need to be calibrated.

Initial pH of ~25 ml analyte is 8.53
Try to bring pH to about 6.3

Using 50 ml portions of 1.0 M HCl
50 ml → pH = 7.00
Approx 110 ml brought pH to 6.34.

n = 34 = 10 ml calibration.

Vol = 20 ml pH = 6.50 n = 11 Δn = 12.
@ n = 17 pH stabilized @ pH 6.44
Therefore we may have had a crossover @ < 6.34.
Try again. Acidify to ~6.1 OK pH = 6.08

Vol = 20 ml pH = 6.23 n = 80
pH stabilized @ 6.13 @ n = 15

Vol = 20 ml pH = 6.34 n = 100+
pH stabilized @ 6.31 @ n = 64

some lemons going on all the time, as usual.

We now have two different set ups going for titration, one w/a low volume, high accuracy burette and the other a high volume, coarse accuracy burette. You see that they both have their place and need.

Next, the detection of calcium is presenting new challenges. The role of pH & its buffering capacity of Ca complexed by EDTA is under observation. The external addition of acid & base near the equivalence point is under observation. The difficulty in surpassing the equivalency point due to same apparent buffering capacity is also under observation.

Coarse titration examinations are now more appropriate and helpful.

pH preparation of the analyte, i.e., proximity to the equivalence point, is an important factor in the success and efficiency of the titration.

Let's try $Ca(OH)_2$ again. Our estimated unadjusted equivalency point was @ ~6.5 to 7 pH. I am going to try something different here. I am going to add some Ammonia to the EDTA titrant.

The unadjusted pH of the 0.01M EDTA titrant is ~7.02. I would like to raise this to ~9.0. Essentially to act as an assist in the reaction.

I added 50ml of 10M KOH to the titrant (~250ml) This raised the pH to ~9.5. I like to live. Let's try it.

Now, the pH of our analyte ($Ca(OH)_2$) solution is ~8.7 So it will be acidified to a pH of ~5.5 3 drops 1M HCl produce the effect. We now have a pH of the analyte @ 4.63. Here we go.

Vol = 20ml	pH = 6.05	Λ = 80	$\Delta\Lambda$ = 14
Vol = 20ml	pH = 6.55	Λ = 82	$\Delta\Lambda$ = 14
Vol = 20ml	pH = 7.20	Λ = 78	$\Delta\Lambda$ = 14

Well, guess what. We have a peak reached @ pH = 7.035

Adjusted pH = 1.19(7.035) − 0.27 = 8.1 = dpH/dV

vs Theoretical 7.5 = .110

I would say we have it. There is no competition to $CaCl_2$. Continue on material.

Vol = 20ml	pH = 8.18	Λ = 82	$\Delta\Lambda$ = 14

Ok. Here is our actual final result.

Our maximum peak actually occurs @ 7.76

$\frac{dpH}{dN} = .012$

There is no real competition other than an unstable short lived peak @ pH ≈ 5.5
There is not enough data to verify the point.

However, the peak @ unadjusted pH of 7.76 is definite.

Adjusted pH = 1.19(7.76) − .27 = 8.96

The theoretical value is ~ 7.5 so then is certainly high than I like. It also brings us into range of Sr^{2+} & Mg^{2+} which will only confuse the matter further.

We will repeat the test.

The main issue here, however, is that the ratio of managing the pH of BOTH the titrant and the analyte PRIOR to titration looks to be very sound.

The appears to be the only means by which the titration can be successful.

Now, the next point of observation here is that the volume required for success was far too high. You would like to succeed w/in the equivalent volume of ~25 ml instead of 80 ml.

To do this, ~~two~~ 3 approaches could be taken.

1. Increase the base or the analyte to allow the reaction to proceed faster.

2. Set the pH of the analyte closer to the equivalence point.

3. Weaken the analyte solution.

Lets use methods 1 & 3, & a little bit of #2.

titrant
1. Raise the pH of the analyte closer to 11.0
2. Use 1 mL of $Ca(OH)_2$ instead of 2 ml in 25 ml total
3. Set the pH of the analyte closer to 6 vs 5.5 if possible.

Run it again.

Your method development here, however, requires smart pH management of the titration as a critical factor!

I have
1. Added 50ml additional 10M the KOH to the titrant.
2. I have used 1 ml of the $Ca(OH)_2$ instead of 2ml with all the total vol of ~25ml.
3. The initial pH of the analyte was 8.17 I will try to titrate to pH ~6.

Adjusting the pH close to the equivalence point is a delicate matter involving 20-50 ul. I now have the pH @ 6.4. Here we go.

Vol = 20ml pH = 8.46 n=79 $\Delta n = 14$

Ok, we have a definite peak. The titration was highly successful & the titration was contained w/in 30 ml instead of 80ml.

Our peak is @ pH = 7.82 unadjusted
Our previous trial was @ 7.76 so we agree again completely.

This tells us that our pH calibration regression definitely has some error in it and that the pH is actually quite accurate in this range.

pH 7.8 vs theoretical 7.5 and the glass electrode is sensitive to Ca^{2+} with no real competition.

pH Calibration

$PH(adj) = .112pH^2 - .174pH + 2.52$ $r^2 = 0.92$

We now have Ca +2 identified w/ calibrated pH curve

Unfortunately, our pH regression curve dictates that we use

$(1.19)(7.8) - .27 = pH\ 9.0$

Success now with Ca+2

PH = .112MEAS^2 - .174MEAS + 2.52

Which again introduces Confusion into the picture as it now suggests Sr +2 or Mg 2+ are serious candidates & nothing could be further from the truth. At the very least, we would like to add another data point to our pH calibration curve:

Meas: pH = 7.8
Actual pH = 7.5

If does seem like working w/ the higher volume burette will generally be easier.

Our pH calibration data is

Measure	Theory		pH 8.0 estimated vs 10.0 w/ Ca Mg2+ & Sr2+
9.51	12.6	Bleach	Clenches it.
7.54	8.3	Baking Soda	We have it
3.90	2.4	Vinegar	now.
10.48	11.6	Ammonia	
1.60	3.1	HCl	
add 7.0	7.5	Ca2+ EDTA titration	

We now have a quadratic calibration curve

Using pH=7.8 $Theory = 0.112pH^2 - .174pH + 2.52$ $r^2 = .92$ $\sigma = 2.35$

So our case is. $pH = 8.0$ (adjusted)

Good work.

And there is a big improvement. 8.0 vs 7.5 theoretical Adjusted

Jun 29 2018

Let's look @ Mg now.

Molecular wt of $MgSO_4$ is : $MgSO_4 \cdot 7H_2O$
246.48 gms / 1000 ml

0.1M = 24.65 gms / 1000 ml
0.01M = 2.46 gms / 1000 ml

.01M = 0.615 gms / 29 ml H_2O let's use these w/ distilled water

MW of Calcium Disodium EDTA is 374.27 gms/mol

so a 0.01M solution is 3.74 gms / 1000 ml

One of the important lessons we have relatedness w/ EDTA titrations is that the pH of BOTH the titrant and the analyte must be carefully considered and managed (ie, adjusted properly) before proceeding w/ the titration.

Example: Ca^{+2} $pK_{Ca^{2+}}$ point is ~ 10.0
Mg

The critical pH of our $MgSO_4$ analyte is 7.41. It is best to bring it up to ~ 8.75 to 9. The pH of the titrant (EDTA) has also been adjusted to bring it to about pH 11.

Ok, we have set $MgSO_4$ analyte to 8.13.

Initial pH: 8.73
Vol = 20 pH = 8.91 n = 78
No peak apparent.
Vol = 20 pH = 9.01 n = 90 Δn = 15
No peak apparent
Analyte site idle for ~ 3-4 hrs.
pH has now regressed to 8.76. Continue
Vol = 20 pH = 8.84
No peak apparent. Continue
Vol = 20 pH = 8.90
Everything here says that the pH of the EDTA titrant is not high enough. Check and repeat.

Sure enough, the pH of the EDTA titrant was only ~ 8.5
Not sure why, but I will raise it again close to 11-12.
Recall we need to go past pH of 10.
I can only get the pH up to about 10.3,
I will use this. 10.3 unadj ≅ 12.6 adj - OK.

Next, the initial pH of the analyte (0.01M $MgSO_4$)
≅ 7.44. Let's raise this to approx 8.0 unadj (≅ 8.3 adjusted). OK, it is sufficient @ pH 7.44

Vol = 20 ml pH = 9.52 n = 80 Δn = 14 15
We have a strong peak @ ~ pH 8.2 unadjusted
This gives an adjusted pH of 8.6

This is definitely a bit low to positively identify Ca^{+2} ion only enough to suggest it as a candidate.
This occurred @ n = 22 so these results are a bit coarse.

In this case, we see that the pH of the titrant was likely a bit too high. We could ~~titrate it~~ weaken the pH a bit and go again. $dPH/dN = .069$

Since our peak was @ 8.2 we can acidify the analyte a bit and bring it closer to ~7. We have also ~~weakened the~~ lowered the pH of the EDTA titrant somewhat.

This should slow the process down a bit
Initial pH = 7.05
Vol = 20.0 ml pH = 9.52 $n = 83$ $\Delta n = 15$

We do have a peak. The titration is still too fast. $dPH/dN = .105$

Our ~~titrate~~ titrant is 7.8 leading to an average of unadjusted pH of 8.0 or an adjusted peak of 8.3 when it should be pH 10.

This is therefore another data point for our calibration curve.
Mean 8.0 Should be 10.0.

	x (meas)	y (theory)
Bleach	9.51	12.6
Baking Soda	7.54	8.3
Vinegar	3.90	2.4
Ammonia	10.40	11.6
HCl	1.60	3.1
Ca^{2+} EDTA	7.8	7.5
Mg^{2+} EDTA	~~8.0~~ 8.1	10.0

Now our calibration curve is
$pH(theory) = .090\,meas^2 + .107\,meas + 1.99$
$r^2 = 0.90$
$\sigma = 2.24$

.09 .107 1.99

Keep tempering the solutions.
Lower the pH of the analyte to about 6.0 now.
Initial pH ~ 5.48
Vol = 20.0 ml pH = 7.75 n = 89 Δn = 15
Vol = 13 ml pH = 8.31 n = 60 Δn = 15

If you change the strength of the titrant you have a moving target. You cannot do this.

Ok you cannot go changing the pH of the titrant mid stream. Our conditions are.
Initial pH ≈ 6.36 Titrant pH ≈ 9.4
Ok there was a smooth run.
Vol = 20.0 ml pH = 9.09 nice & even n = 96 Δn = 15

We definitely have a max, but it is much lower than expected. pH = 7.5 dpH/dV = .067

The data looks smooth but the result does not seem reasonable in the slightest.
A pH of 8.3 is acceptable. A pH of 7.5 is not.

There is certainly some question here about pH Coda Calibration.

We check w/ pH paper

	meas	Actual
pH paper	9	9
pH paper	10	10
pH paper	7.3	7
	~~3.48~~	~~2.4~~
	1.60	3.1
pH paper	3	4.1 Vinegar

10.7 to 12 pH paper - ammonia
~~+0.40 to~~

$pH = .116 Meas^2 - .41 Meas + 3.09$
$r^2 = 0.97$
$\sigma = 0.62$

We have some real questions here, especially about

1. pH Calibration
2. Why a pH for Ca^{2+} being detected when we should be detecting a pH for Mg^{2+}? (7.5 vs 10.???)

I notice that the ΔpH is very responsive near pH 9.4 and then becomes unresponsive immediately after.

This says that our pH calibration is much closer to reality than previously determined.
Initial pH = 7.81
Vol = 20.0 mL pH = 9.75 n = 96 ΔV 0.15

I am noticing something. The optimum part of the reaction may not be the maximum slope of the pH.

It appears that it is actually when the pH levels off sharply. This would indicate completion of reaction.

In this case, we have a very pronounced sweet spot @ a pH of 9.46, right where it should be for Mg^{2+} (~10).

We also have a steep rise, however, in pH before this point is reached.

Our pH calibration may be much closer than thought.

For now, use the relation

$pH(theory) = 0.116\,meas^2 - 0.41\,meas + 3.09$

$r^2 = 0.97$

$\sigma = 0.62$

It may well be that pH paper is the best way to calibrate.

In that case, it seems necessary to have a strong base in the EDTA titrant. That makes a lot of sense.

pH of titrant is now about 10.5. That is good. (Ideally ~ 11.6 – even better)

.116 -.41 3.09

Ok, I have completed a pH calibration w/ pH paper, and I am getting excellent results.

$pH = \underset{0.062}{\cancel{0.101}}\,meas^2 + \underset{0.299}{\cancel{0.403}}\,meas + \underset{1.27}{\cancel{4.43}}$

$r^2 = 0.99$

$\sigma = 0.22$ That is so.

Based upon data

Meas	pH paper Test	
9	9	EDTA Sample
10	10	EDTA Sample
7.3	7	EDTA Sample
4.1	3.5	Vinegar
10.7	12	Ammonia

The new pH Calibration Curve shows that our meter is working actually quite well. The adjustment is actually quite small and in many cases could actually even be disregarded.

This puts an entirely different spin on the interpretation of results.

We may no longer actually be looking for the maximum but for the

COMPLETION of the reaction, ie a sharp drop off in the pH after successively climbing to an elevated value.

—

Ok, here we go. We know the pH meter is actually supremely clear now. We believe we are now looking for the completion of a reaction rather than a maximum change

Initial pH = 7.6 (Looking for ~ 10)
pH of analyte = ~11.0

We can definitely see a rapid reaction that takes place that then ebbs @ pH ≈ 9.4.

This is an entirely different interpretation.

Looks like good work here and a breakthrough

Vol = 20 ml pH = 9.79 n = 88 Δn = 15

Now, looking @ the data differently, we see that the leaped change in pH settle down @ pH = 9.59 - 9.65

Obs pH = ~~9.04~~ 9.93 vs ~10 theoretical for Mg 2+.

I think we have found what we are looking for.

Now our best indicator here was actually not the slope so much. It was when dPH / dN → Ø

The computational detection scheme for this is a little complicated.

It seems to be when

$$\frac{dpH}{dN} * \frac{dpH}{\Delta N = 1} \rightarrow \emptyset \text{ (approaches zero)}$$

This seems to identify when the reaction is over.

We now back up & reexamine

Fe 2+
Mn 2+
Ca 2+
Mg 2+ We definitely seem to have Mg +2

We also notice a whole complex has been formed w/ Mg+2 and EDTA @ pH = 10

Jun 29 2019

Back to Fe+2 detection now.
pH of EDTA titrant is 8.00 7.7
This should be adequate.
$FeSO_4 \cdot 7H_2O$ MW is 278.00 gms/mol

.01 M solution is therefore 2.78 gms/1000 ml.
or 0.10 gms / 250 ml Acidify it slightly to reduce oxidation.

Initial pH of $FeSO_4$ analyte is ~~6.3~~ 6.09
Acidify to ~3.5-4
I will also switch to volume intervals of 15 ml as $\Delta n = 13$
Initial pH is 3.74
Vol = 15 ml pH = 3.99 n = 71 ΔpH = .25
no peak evident.
Vol = 15 ml pH = 4.26 n = 80 ΔpH = .27
no peak evident.
Vol = 15 ml pH = 4.04 n = 88 ΔpH = .58
pH now 4.95
This shows that reaction is still proceeding to the point.
Vol = 15 ml pH = 5.34 n = 75 ΔpH = .50
This shows the max reaction should have occurred here

We have a clearly identified peak (cumulative reaction) @ pH = 5.16 vs theoretical 5.1.
Our adjusted pH is 4.46 which does not appear here. This shows that our pH calibration error is within range of the meter study.
There may be no real advantage to using the calibration curve except @ extreme ends

of the pH scale.

We have, therefore, identified Fe^{+2} with high certainty and accuracy.

Our volume was a bit high w/ the titrant so, it would be best to increase the pH of the titrant to about 9. A by 3-4 pH would be helpful.

I would like to
1. Attempt separation of mixed Fe^{+2} & Mn^{+2}
2. Increase pH of EDTA titrant to ~9.

Incidentally, we get a clear transparent complex here w/ a light yellow green color. The color will also be distinctive and useful w/ various metals.

VIS Spectrometry can be used here also to further validate

Mn^{+2} + Fe^{+2}
Initial pH of titrant 7.1 Increase to ~9. Done.
Initial pH by analyte = 6.4 Acidify to ~4. Done.

Notice solution begins to clear immediately w/ addition of EDTA.
Vol = 15.0 ml pH = 4.70 n = 71 Δn = 13
Vol = 15.0 ml pH = 6.46 n = 78 Δn = 13

We pick up a drop on lines 20 & 23.

Using midpoints of the Δx interval seems to be the most appropriate.

Line 20: $pH_1 = 4.94$ $pH_2 = 5.17$ $\bar{x} = 5.06 = 5.1$ [5.1]
(adj pH = 4.4)
Theory played a the in Fe^{+2}.

Line 23: $pH_1 = 6.02$ $pH_2 = 6.25$ $\bar{x} = 6.1$ [5.6]
(adj pH = 5.4)

Using either set of values, we end up w/ the same conclusion. We have identified both Fe^{+2} & Mn^{+2} successfully. Very good work.

Adjusted value of pH 4.4 would also lead to consideration of Co^{+2} & La^{+2} but the color of the complex would certainly dictate Fe^{+2}.

Our best pH value may actually be a mean value between the meter directly & our regression curve.
This leads to
Fe^{+2} pH estimate @: 4.8 vs 5.1 $\Delta = 0.3$
Mn^{+2} pH estimate @: 5.8 vs 5.6 $\Delta = 0.2$

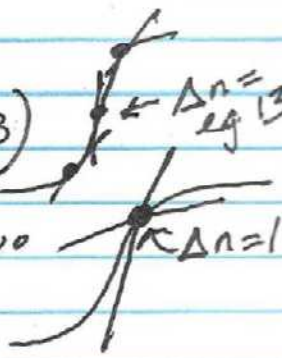
This is quite good work and does effectively identify & separate the presence of Fe^{+2} & Mn^{+2} in our solution.

Color of complex formed is also very useful information.

Our computational method of identifying the endpoint of the reaction is an relatively one.

We are looking @ two things:

1. The slope (dpH/dN) w/in a Δn interval (eg, $n=13$) (average)
2. The slope at the crossover point between two Δn intervals (i.e. $\Delta n=1$)



* The product of these two factors will produce a minimum and represent a sharp dropoff (local) while the reaction is proceeding. The method shows itself to be very effective in the absence of graphical representation (which my titrator does not have.

There is an ENDPOINT determination point that has been developed which is quite effective as demonstrated in the separation of close pH values nevertheless representing two different ions.

—

Ok, great work. Our remaining check is that by Ca^{+2} and then I would like to look at Al^{3+} ???

—

Now we will look @ Ca^{+2} Detection pH is approx 7.5. Drop pH to ~5. Leave EDTA pH @ ~10.5.

Vol = 15 ml pH = 7.02 n = 12 Δn = 13
pH → 7.09 during idle
Vol = 15 ml pH = 8.06 n = 75 Δn = 13

We have an endpoint at pH = 7.30
Adj pH here is 6.8.

7.3 is, once again, extremely close to theoretical pH of ~7.5.

We do have Ca^{+2} identified here.
Once again, there simply may be no need for a "Calibration pH curve". Our meter, ESPECIALLY IN THE MID PH RANGE, seems surprisingly accurate.

I have now succeeded in the detection and identification of

1. Fe^{+2} light green
2. Mn^{+2} no color in low concentration?
3. Ca^{+2} no color in low concentration
4. Mg^{+2} white precip?

and a combined solution of Fe^{+2} & Mn^{+2}.

Good work & methods have developed here.

I have just determined that I can easily graph my computed detection factor for the Casio Calculator - spreadsheet.

This is invaluable. I can now see the results of my titrations, and even more importantly, my own computations graphically.

Another major aspect to the process is in place now. Great!

The advanced calculator spreadsheet that has been developed here is now invaluable and essential to the titration procedures that have been developed.

Al^{3+} detection pH is ~ 4.2.

Let's see what form of aluminum we can & would like to use.

Aluminum Sulfate - we have MW 342.13 gms/mol

$$\frac{3.42\text{ gm}}{1000\text{ ml}} = \frac{x}{250\text{ ml}} \qquad x = \frac{0.869\text{ gms}}{250\text{ ml}}$$

$Al_2(SO_4)_3$ analyte pH is 5.01. Lower to ≤ 3.

Acidified to pH ~~3.09~~ ~~3.01~~ 2.98

Vol = 15.0 ml pH = 3.00
No significant pH change
Not enough Change.

pH of analyte does not need to be ≤ 3.5

Set pH to 3.5

Vol = 15.0 ml	pH = 3.62	n = 71	ΔpH = .32
Vol = 15.0 ml	pH = 4.10	n = 76	ΔpH = .28
Vol = 15.0 ml	pH = 4.65	n = 83	ΔpH = .55

We have a definite endpoint @ pH = 4.09

Excellent. Theory is 4.2.

Here is a case where the pH regressed while sitting idle, instead of climbing as usual. This indicates the reaction had completed.

This means that we have now successfully detected and identified

1. Fe^{2+}
2. Mn^{2+}
3. Ca^{2+}
4. Mg^{2+}
5. Al^{3+}

and a mixed solution of Fe^{2+} & Mn^{2+}.

HEPA FILTER ANALYSIS

June 30 2019 — METALS - INORGANIC - PROTEIN

I plan to do some investigative analysis of an extract from a received HEPA filter. This same HEPA filter material was that used to develop the cultures last year.

I have made an extract w/ Conc. H_2SO_4.
I have made an extract w/ Conc. KOH.
I have filtered the extracts in each case.
I have combined both extracts into a common solution. I will concentrate that solution further by evaporation.
Final volume of the concentrated process will be ~ 200 - 250 mL of solution.

I have conducted colorimetric tests for Fe ions.
Both Fe^{+2} & Fe^{+3} produce a positive colorimetric test.

It is an interesting situation when you look at the periodic table. The periodic table is vastly dominated by metals, especially w.r.t. the more common elements.
In one way or another, between the metals and the metalloids we are looking @ a range of up to 75% of the periodic table is occupied by these elements.

Metals are indeed an extremely important base of chemistry to understand.

The reddish color for Fe+2 detection (1, 10 Phenanthrolin) takes a while to develop and clarify but it most especially does, especially after setting for about 1 hour.

Maximum absorbance was @ 510 nm, exactly as what we saw in previous tests for Fe+2.

Fe+3 test (weak concentration here) gave a max absorbance @ 444 nm.

Very different positive colorimetric reactions here.

The data show the value of qualitative "wet" chemistry testing for ions. It is relatively simple in many cases and usually fairly configurable as well. It is a good start.

There is no real need to titrate for Fe+2 & Fe+3 in the case unless you need to get involved in concentrations.

The next observation: heating the acid-base extractions (now combined) at a moderate temp of ~ 65°C denaturizes the protein, or at least a protein found with in the solution.

It can also be easily observed under the microscope and it is clear CDB protein complex formation. This also has the effect of clarifying the extract solution.

With some attention, the protein complex material (now insoluble at the current pH) should be able to be collected and concentrated.

A question that arises is, when the material has denatured & solidified, does this change the detectable metal ions that were or are in solution..?

The pH of the current combined solution is ~2.4. Therefore it is highly acidic. This tells us that we have an insoluble form of the protein complex that is denatured and that it exists in the form in a highly acidic environment.

After evaporation and concentration, later neutralization of the solution and its effect upon the protein complex will be made.

Recall the sample here under analysis is that of a HEPA filter extract.

I need to know if the iron ions are still available in solution or not.

—

Ok, the first result is that Fe^{+3} is still very much in solution. Very much a positive colorimetric test result.

I will let Fe^{2+} test out a while to see if it develops further. There is a positive result but it is very weak.

—

You now have 3 different methods of testing for inorganics

1. Qualitative chemical testing - colorimetric, reactive
2. Titration
3. Electrochemistry

They are all portable/mobile methods w/ reason. Not bad.

—

What we see here is that heating of the (still) acidic extract form increased the concentration of Fe^{+3} ion and decreased the concentration of the Fe^{+2} ion.

Both forms are still present but Fe^{+3} is in much higher concentration now than before heating.

A concentrated NaOH application also produces a strong visible brown precipitate, another form of qualitative testing that validates the existence of Fe^{+3} (in this case) in the extract.

At this point, there is no qualitative colorimetric/precipitative test that indicate the presence of Al^{3+} or Ca^{2+} @ the concentration labeled.

Visible spectroscopy also confirms the Fe^{+2}/Fe^{+3} change in concentration assessment.

—

Titration has the disadvantage that a fair amount of volume of titrant is required to run a single titration. There is a distinct disadvantage be such volume of material is frequently not available.

Electrochemistry is FAR superior in this regard.

—

Here are a couple of things that we see.

1. The extraction is extremely acidic. About equal volume of 10M KOH was required to neutralize it.
2. Neutralization has caused the Fe^{+3} to precipitate out. It is surprising how much iron precipitated out of the extraction.

The remaining filtrate is now very clear along w/ the previous centrifugation of the protein complex / CDB that has isolated (amount is small).

Therefore we know that in the HEPA filter we have

1. CDB Protein Complex
2. CDB
3. A very high iron content.

We know that we have KCl & NaCl salts in the solution due to the extraction and neutralization process. Also sulfates.

These are suitable for IR analysis as well as ~~Electrochemistry~~.

IR analysis: There are no similar matches of reasonable conformation ($R < 0.70$) in the Ventura database to this plot. There are no hydrocarbons showing in the spectrum.

Dominant Peaks are: (expect sulfates?)

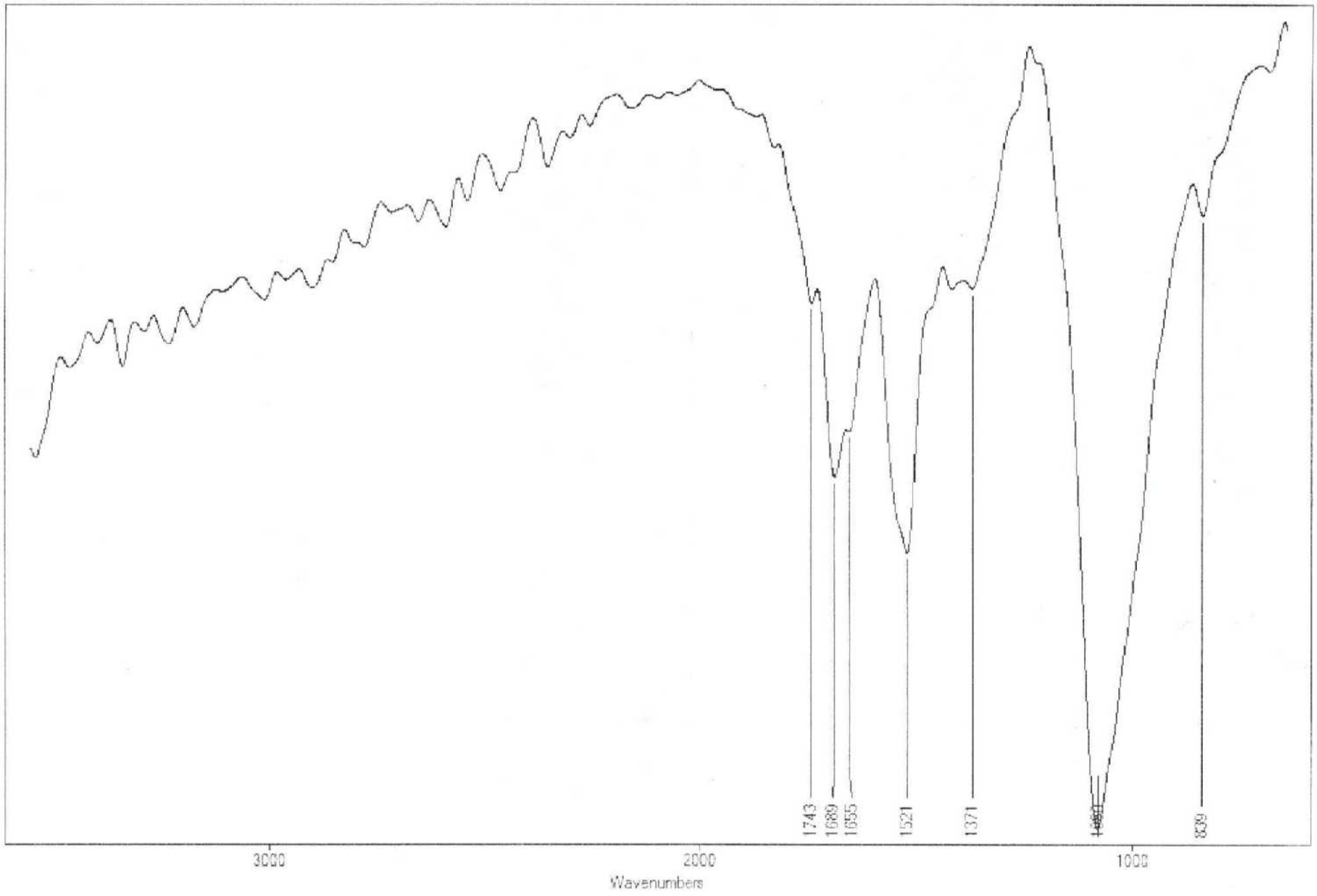
strong	1689 cm^{-1}	Amide 1610-1690 $CONH_2$ - primary Secondary Amide (1670-1700) CONHR
strong	1521 cm^{-1}	N-H amide (1510-1550) C=N Pyrimidines (1520-1580)
very Strong	1080 cm^{-1}	P-O-C very strong (1030-1090) Phosphoric Este

HEPA Filtrate – Isolate – Acid Base Extraction – Neutralized
Protein Denaturation Filtered – Iron precipitation Filtered.

HEPA Extracti

printed on 08/

HEPA Extraction - Acid - Base - Neutralized - Protein Filtered - Iron Removed - Jun 20 2019 - 01.spc: Synthetic spectrum by averaging. See audit trail for details.



printed on 06/20/2019 17:47:45

With SDBS, there also are simply no good matches @ this time.

One thing that is happening is that NO compounds are showing up in almost every candidate - only one exception that has a N compound vs NO.

All of the compounds in SDBS exhibit hydrocarbons which mine do not.

Ihr says that we do have a nitrogen based compound here

Actually we are seeing N and C=O frequently.

—

Well we see amide all over the place and phosphorus ester is also a strong candidate.

* There is such a thing as an "amide of phosphoric acid", also an "amide of phosphoric acid".

From Notes of June 14 2019:

Protein Reagent - Best Results

~~Old~~ Protein Reagent:

1. Stock solution of Red Dye is:

300 µl of Ritz Cherry Red Dye in 10 ml H_2O
(dye is too thick for pipette flow, add 1 drop of H_2O)

2. Reagents:

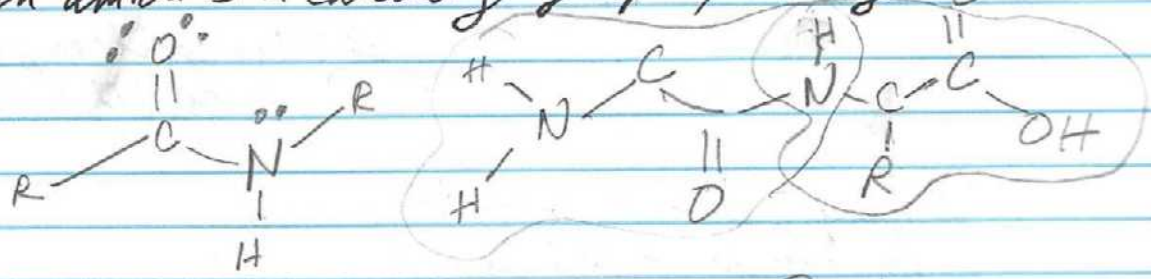
1. 2ml of H_2O (3 is not needed / desired)
2. 100 µl of stock red dye solution
3. 1 drop 10M NaOH
4. 2 drops 0.5M $CuSO_4$
5. Pinch of Cream of Tartar (tartaric acid)

=

The colorometric test for protein fails. However, we have strong IR evidence of an amide functional group.

Can you have an amide w/out having a protein?

An amide is a carbonyl group w/ Nitrogen

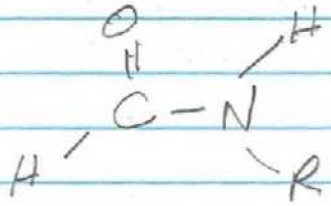


Amide

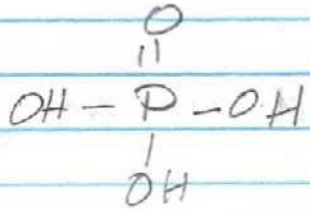
Dipeptide Bond

Amides are $RCONH_2$ phosphoramidic carboxamidic

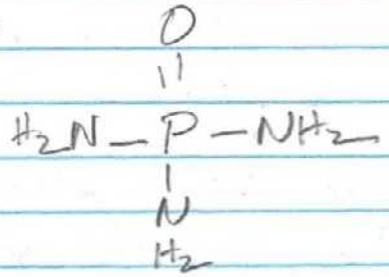
Our best model is $CONHR$



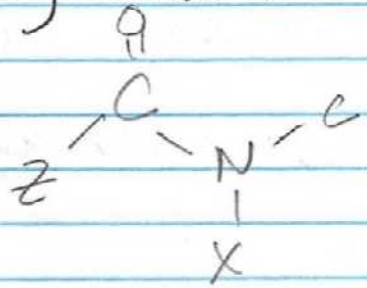
Phosphoric acid is



Phosphoramidic is



Secondary amide:



X = any atom but C and usually hydrogen

Z = any carbon group or hydrogen

A "group" is a collection of atoms that do not form an entire molecule or ion.

if we enter SDBS w/ the 3 absorption peaks but just Phosphorus into the search only 1 entry shows up

It is a monophosphate sodium salt.

absorption @ 1080 cm^{-1} = 10 excellent (10 out to 4th)

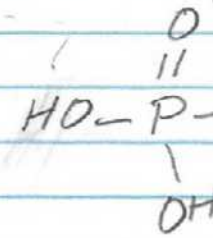
absorption @ 1521 ≅ 25 also excellent

absorption @ 1609 ≅ 4 also excellent

These three absorption peaks are an excellent match and it is the only compound w/ phosphorus. Looks like a pretty decent match to me.

Notice there is no amide in the compound.

Monosodium phosphate is NaH_2PO_4



Jul 19 2019 Stevensville, MT area
• Bass Creek

I am studying some statistical testing methods.

t distribution varies from the z distribution as it is adjusted to a sample standard deviation vs a population σ.

$$z = \frac{x_i - \bar{x}}{\sigma} \qquad t_{n-1} = \frac{\bar{x} - \bar{x}}{(\sigma_s/\sqrt{n})} =$$

Example for t computation:
$n = 1000$
$\bar{x} = 2.5$ hrs
$\sigma_s = 0.5$ hrs

$t =$ $\frac{\sigma_s}{\sqrt{n}}$ is a factor that you multiply the z score by.

In our case, this factor is $\frac{0.5}{\sqrt{1000}} = .016$

and $2.5 \pm 1.96(.016) = 2.5 \pm .031 = 2.47$ to 2.53 @ the 95% level.

t distribution is used when the sample size is ≤ 30 and σ_s is known, which is actually usually the case.
z distribution is used when the sample population σ is known, which is almost never. Do keep that in mind.

How to use a t table properly is a good start here.

Can will compute & graph the various probability distributions. For example, if you set

$X_i = 1.6449$ (as if it were a z score) (ie, 90% level)
$\bar{X} = 0$ (also being called "u")
$\sigma = 1.0$ (not $\sigma_{\bar{x}}$)

This is the Normal Probability Distribution Curve. Not the same

And compute p, you will get $p \cong 0.10 = 90\%$
You can also draw the graph and see that you are operating @ the 90% level.

If $X_i = \cancel{0.10}\ 0.68$
$\bar{X} = 0$
$\sigma = 1.0$ $p = \cancel{0.34}\ p = 0.32 = 68\%$

If we use the cumulative normal distribution curve we get what we expect.
Use variable mode we last made
Lower = -1 (ie, there a z score)
Upper = +1 (ie, there a z score)
$\sigma = 1$
$\bar{X}(\text{or } u) = 0$

We compute $p = 0.68$ ie 68% w/ $z = 1$ ok
It also draw the area as a shaded curve.
That a our normal distribution curve.

Very smart graphic computer.

Normal C.D.
Lower = -1.6449
Upper = +1.6449 $p = 0.90 = 90\%$ as expected
$\sigma = 1.0$
$\mu = 0$

The normal probability distribution curve p is the magnitude of the curve, not the integral.
The cumulative distribution curve represents the integral of the curve. That is what we want.

Ok, now let's look @ the t distribution. (Cumulative).
Ok, that is a very interesting distribution.

Here is what you specify:

The upper "t" score
The lower "t" score
The degrees of freedom $(n-1)$

That is really quite interesting. It shows you that for a small sample (ie $n \leq 31$) your t score will need to be higher than the analogous z score to attain a certain confidence level.
Recall that you will know σ_s but you will not know σ.
Example
$n = 6$ & degrees of freedom $= 5$

90% confidence is attained w/ $t = \pm 2.02$ vs 1.6449

That is a very significant difference

And, the difference is usually considered conveniently ignored in the more normal study of statistics. It makes perfect sense that it would be since there is more trial and error required w/ the degrees of freedom input requirement.

You should definitely adjust your thinking in the as soon as possible, it will yield much more valuable analyses for small samples.

If $n \leq 30$ you should also be using the t cumulative distribution instead of the normal distribution.

We know now that the t distribution is adjusted by the factor $\frac{\sigma_s}{\sqrt{n}}$ (z score) $+ \bar{X}$

The graphing & computation of this curve is a very valuable asset.

We should be much less likely to use the normal distribution curve w/ small samples now, indeed you understand that it really NEVER should be done.

Then now brings up to discussion your development of the logarithmic distribution curve that you developed in earlier years to handle the "long tail" curve that arose in the generalized analyses of years past.

You have to wonder now, knowing that in general small samples are quite commonly used, and that use of the t distribution may have alleviated or eliminated many of your concerns.

This is a very intriguing and interesting question.

The use of the logarithmic distribution curve that was developed in original research seems to eliminate many if not most of the problems that occur w/ the use of small samples.

A very interesting question indeed.

Jul 20 2019

Okay, now looky @ Analysis of Variance (ANOVA)

Example: How does a drug affect blood pressure..?

The "factor" ~~(i.e. independent variable)~~ is the drug dosage.
A second factor will be how many times a day the drug is taken.

Sorry, let's back up. The above is a 2 way Anova.
Let's start w/ a one way anova.

The t test is a precursor to the oneway Anova when we only have two population means.

Male watermelon spitters : 56.5" $n=10$ $\sigma_s = 8.45$
Female watermelon spitters : 47.0" $n=10$ $\sigma_s = 9.02$

Can we take care of the data with the 2 sample t test (variable mode).

One question comes up as to what it means to "pool" the data or not. Unknown answer @ this point.

In the case $p = .039$ the are significant @ 95% level.

The sample sizes do not need to be the same.

Now, what if we had numerous sample groups, such as by age, for example instead of just two groups such as male & female.

Then we have Anova (One Way Comparison)

The data is:

6-8 yrs	9-11 yrs	12-14 yrs	15-17 yrs
38	38	44	44
39	39	43	47
42	40	40	45
40	44	44	45
41	43	45	46

This data all gets sorted into two columns

Ok this is what is not @ all intuitive. So it is of form

List 1	List 2
1	38
1	39
1	42
1	40
1	41
2	38
2	39
2	40
2	44
2	43
:	:

This is unusual but that is the method.

Results $p = 1.3E-3$

Significant @ 95% level.

Assumptions are:

1. Data sets are independent
2. Data sets are normally distributed
3. Variance of the data sets are equal.

Now, that is a real handful of assumptions

Sample sizes do not have to be the same.

Evaluation of the three assumptions is an interesting challenge in its own right.

The data needs to be entered into 4 separate lists (not the unusual 2 list format we have for one way anova) to begin the examination. We do not have this.

Just maybe we can get away using the "Normal probability plot" on the Combined list #2 format and see that it is generally linear in form. Maybe this suffices for the normality determination question.

—

Do we have a low p value. Now we need to examine how to find out which means are different from one another not just that they are different. This is a weakness of the Anova method.

—

The fit of the Anova model is an important follow up. Calco does not provide R^2 but it does provide a MSE.
In our case, MS(MSE) = 3.55"

This is apparently not too bad and will lead to an R^2 of 54.0% (we do not know how yet).

A value > 80% is considered favorable.

I think that you can get a rough idea of the success of the model by computing:

$\frac{MSE}{\bar{X} \text{ of data set}} = \frac{3.55''}{42.35''} = 8.4\%$

Note: Maybe we want $\frac{\sqrt{MSE}}{\bar{X}}$

we do not want a "squared" error

which is apparently only a fair result.
You have to consider that 30 ∋ ~ 25% error when we know is not great
I suspect that you would like to see a $\frac{MSE}{\bar{X}}$ on the order of ~ 3% or less.

—

Next we need to go after "multiple comparisons" to sort out the means.

There is also somewhat of a loaded issue of many different strategies of varying advantage & disadvantage.

I do not think Casio has any built in "multiple comparison strategies.

—

Ok, the Classpad 400 calculator does the 1-way Anova very easily & much more intuitive. Each sample set (up to 6 samples) are entered in each list separately - much more intuitive.

The results are presented in a straightforward fashion.

Once again, w/ the Casio Classpad 400
we get a

$p = 1.38E-3$

and a
$MSE = 3.55$

We also get very cool box plots of each sample set very easily on the Classpad 400.

What we do not get, however, is Multiple Comparisons.

We can however get the individual t test results quite easily and these are good. (Because entry format by sample set is sensible here).

List set	p Value
S1 - S2	Ø.575
✓ S1 - S3	.022
✓ S1 - S4	3.8E-4
S2 - S3	Ø.138
S3 - S4	0.067

Ok, this method will work.
Ranking of Δ means is therefore:

S1(6-8yrs) - S4(15-17yrs) 3.8E-4 > 95%
S1(6-8yrs) - S3(12-14yrs) .022 > 95%

Conclusion:

Older kids can spit farther than younger kids. Not exactly a total surprise, but it does demonstrate the method well. And use of Casio Classpad 400 is intuitive here.

Now, w/ respect to multiple comparisons, we have also made much progress here.

Our method being used here, with Anova first being conducted and then and only then w/ a significant result multiple t-tests being conducted is called

"Fisher's LSD" (least significant difference) and it is a viable multiple comparison method. Anova must first be done and lead to hypothesis rejection however.

So Classpad 400 does work out here because of data entry format. Casio FX 9750 GII does not exactly do so; it is awkward w/ data entry and would require two separate data entry methods to conduct Anova & then t-test pairs

Casio Prizm calculator apparently also has multiple comparison strategies built in.

We will soon move on to two-way Anovas.

We have one more data set w/ a more interesting case, cell phone usage w/ different age groups and time on the phone.

We get $p = 1.20E-22$ (very significant)
$MSE = 3945$ ($\sqrt{MSE} \approx 63$ min)

	p
S1 - S2	6.30E-11
S1 - S3	5.66E-3
S1 - S4	2.00E-14
S2 - S3	5.29E-9
S2 - S4	4.97E-8
S3 - S4	3.24E-13

Ranked:

		Age Group
S1 - S4	E-14	<19 vs >60
S3 - S4	E-13	40-59yrs vs >60
S1 - S2	E-11	<19 vs 20-39 age

So:
Old people vs young people have greatest difference
Middle age vs old has next greatest difference
Young vs young adults have next greatest diff

The analysis w/ a good calculator, is quick and effective.

Hypothesis is that the means are the same by Case. But Ramsey book uses Ho as the means are the same. This is a point of confusion.

Jul 21 2019

We do know that our computations agree completely w/ Ramsey & examples so it does seem to me that Ho is indeed that the means are the same. I shall keep an eye on this.

Behavior of all 3 Casio calculators is different:

1. fx-9750GII has awkward Anova data entry but it works fine. Mult. pair comparisons would require separate list data entry, however.

2. Classpad 400 very easy to use w/ data entry format and mult. pair comparison panel & tests/are easy to complete. Also good box plots of list data.

3. Casio Prizm apparently has extended multiple comparison tests. We shall see.

Before proceeding, I am interested in how R^2 is determined since Casio does not provide that and it is an important results as to the fit of the anova solution.

I see that my hunch was correct, i.e. $\sqrt{MSE}$ is an important number in this regard. Minitab will designate this as an "S" term, then to be followed by R^2.

S could be regarded as the "standard error" (s) of the Anova model it seems.

We are given that:

$$R^2 = 1 - (SSE/SST)$$

and that

$$R^2_{adj} = 1 - (MSE/MST)$$

so what is MST?

$$\frac{MSE}{MST} = 1 - R^2 \quad \Rightarrow \quad MST = \frac{MSE}{1 - R^2}$$

Assume $MSE = 3.55$ $\quad R^2_{adj} = 53.97\%$ $(= .53973)$

$$\text{So } MST = \frac{3.55}{1 - .5397} \qquad \text{or} \quad MST = \frac{3.55}{1 - 53.97} \quad (\text{NO})$$

$MST \overset{?}{=} 7.712$ The value is not computed or available.

R^2, called the coefficient of determination, is defined as the ratio of the sum of squares divided by

"the sum of squares around the mean".

"Proportion of variation explained by the model.

$R^2 = \frac{SST(\text{between groups})}{\text{Total Error}}$

In Minitab SST is called SS in the factor row.
SSE is called Total in the Total row.

mean error / mean error
$MST = SST/(k-1)$ — k = the no. of treatments
$MSE = SSE/(n-k)$ — n = total sample size, k = no. of treatments.
$k-1$, $n-k$ are the degrees of freedom

So now we know:
$F = \frac{SST/df_1}{SSE/df_2} = \frac{89.15/3}{56.80/16} = 8.43$
this is the F statistic

The bracketed section is how the F statistic is computed. A ratio of mean error quotients.

However, we now know how R^2 is actually computed and it was not obvious. It is

$$R^2 = 1 - \left(\frac{\text{Variance Error Between Groups}}{\text{Total Error}}\right)$$

In our example by Ramsey p.44 165 & 176
← Minitab Error SS
$R^2 = 1 - \left(\frac{56.80}{146.55}\right) = 61.2\%$ + $R^2 = 1 - \left(\frac{142030}{2558040}\right) = 94.4$

↖ Minitab Total SS

The difference noted and the proper determination of R^2 is important. Even Ramsey takes over this issue.

Now let's see what Cario provides:

With Cario, you also have to be careful

In Cario, we need to compute

$$R^2 = 1 - \frac{ErrSS}{(ErrSS + ASS)}$$

Again, it is not obvious here.
Minitab gives the total error.
Cario does not and it must be determined.

I think in Cario we should just ignore the "A" designator, as I think it only refers to Anova.

Actually we see that our Cario determination is actually of the form:

$$R^2 = 1 - \left(\frac{SS}{SS + ErrSS}\right)$$

← This is equivalent to Factor SS in Minitab

← This is equivalent to Factor SS + Total SS in Minitab terms

Although this has been tricky to wade through, we now know how to determine the Coefficient of Determination, or R^2

w/in an Anova model. This is an extremely important term of effectiveness of modeling the variance by the model.

If the value is very low (ie; much lower than 80%, for example) this tells you that you likely need another factor in the model, and this leads us to the 2-way ANOVA situation.

We are in much better position now.

Two Way Anova: Here we have 2 different factors that produce the response.

The general structure is:

Factor A	Treatments Response Treatment 1	Treatment 2 Factor B	Treatment m
Factor A Level 1	B	B Response 2	B Response
Factor A Level 2	B(2,1)	B(2,2)	... B(2, m)
Factor A Level 3	B(3,1)	B(3,2)	... B(3, m)
Factor A Level n	B(n,1)	B(n,2)	... B(n, m)

Casio is somewhat limited as to the size of the matrix it can handle, on the order of 3x6 to 4x4 or so.

Apparently a more common structure would be of the form.

The lists (or columns) themselves denote that these are different levels.

Levels for B	A factor (Levels for A)		(row factor)
	1	2	
B factor (Column factor)			
1	11	14	entered as a 3×3 matrix into Casio
2	21	15	
3	38	62	

This column should not have been included

We get 3 results here. One entry for me a 3×3 matrix.

We get $A_p = 8.64E-3$

$B_p = 1$

$AB_p = 1$

This tells us that factor A has an effect upon the response (ie, the No of times the drug is taken per day.

It tells us that factor B (ie, the dosage) had no significant effect.

And it tells us that the interaction of dosage & time had no real effect.

Our R^2 value is:

$R^2 = 1 - \left(\frac{3920.7}{5638 + 3920.7}\right) =$

(actually ends up being OK - see next page)

Let's go back & understand the error explained with our model.

We explain 3920.7 A
+ 0 B
2E-11 AB
Σ = 3920.7 of the total variance.

Our total variance in the data is 5638
Therefore, according to our earlier analysis, our ratio of the variance explained is

$$\frac{\text{Variance explained}}{\text{TOTAL variance}} = \left(\frac{3920.7}{3920.7 + 5638}\right) = 0.41$$

↑ Note

So our R^2 value = 1 − 0.41 = 59%
which is hardly great

How you define Total Error here is important.
ESS is NOT the same as Total in Minitab.
(in Casio)

—

Let's go back and recheck the R^2 relationship for the 1 way Anova.

—

Ok, it is absolutely true that Casio does not output the TOTAL ERROR that is required to properly compute R^2.

Casio gives the

Factor Error (known as ASS, BSS, etc) but known as SST in Minitab)
and
ESS (also known as SSE in Minitab)

Casio Nomenclature
A_SS, BSS
Err SS

First, to define terms

SST is the variability between the groups
SSE is the variability within the groups

The total variability is the sum of these two variabilities and CASIO does not give them. Minitab does and calls it SSTO, or "Total"

and therein lies the confusion, as well as with the difference in terminology.

As we see we have

in Minitab	in CASIO
$R^2 = 1 - \left(\frac{\text{Factor Error}}{\text{"Total" Error}}\right)$	$R^2 = 1 - \left(\frac{\text{Factor Error}}{\text{Factor Error} + \text{Err SS}}\right)$

Note!

and so Total Error is NOT Given by CASIO.

Ok, let does clarify it further, and you will need to refer to these notes as well as Ramsay's STAT II book to recall & sort it out.

An analysis of variance (2 way) can become much more complicated

I see that we can get very nice box plots (multiple graphs on one screen) w/ Casio Classpad. This can be done in the List Mode by using the graphical icons @ the top of the screen.

The box plots give us a good idea as to what to expect from the ANOVA study.

Let's set up box plots for the ANOVA 2 way study
Done only to a partial degree
There is no graphical output of Anova on Casio

Let's move on to the question of interactions
There are apparently (i x j) interactions possible
There is no example given here by Ramsey except via a Canned Minitab approach. The discussion is lacking here.

I Cannot find any clear examples on subtraction. Let's create our own test case

~~Graphpad Prism~~ is a software program tailored to statistical analysis.

It is NOT the same as the Casio Prizm calculator.

Very expensive software. Not flexible.

Let's create our own example
Can handle a 3x4 matrix in 2 way anova

	Water Temp		
Detergent	Low (1)	Med (2)	High (3)
1	37	56	72
2	45	22	40
3	30	34	29
4	22	27	37

a randomly created data set

Effectiveness of cleaning

Actually:

B factor

Water Temp	Detergent			
	1	2	3	4
1	37	45	30	22
2	56	22	34	27
3	72	40	28	31

A factor

$A_p = 1$
$B_p = 1.833E-4$
$AB_p = 1$

So which is the A Factor & which is the B factor and how do we know?

We know that A has 2 deg of freedom
B has 3 deg of freedom

Therefore A is the Temp Variable
and B is the Detergent variable

Therefore A is the Row Factor and B is the Column Factor.

$$R^2 = 1 - \left[\frac{(6E-11 + 3909 + 3E-11)}{(3909 + \emptyset + \emptyset + 3126)}\right] = 44.4\%$$

Not a good model but it gives us something to work with.

Now, box plots really give you a good graphical sense of the data and Cario Clausen is actually very good @ them.

There seems to be a fairly large difference between List 1 - List 3
& List 2 - List 4

There's a 3×4 matrix. Therefore there are 12 possible interactions. How do you explore all of these?

(3×3)

1-2	Column	4-4
1-3	Row	1-2
1-4	Interaction	1-3
2-3		2-3
2-4		
3-4		

Row Column Interactions

Row 1 - Col 1
Row 2 - Col 2
Row 3 - Col 3
Row 3 - Col 4

But this is 13, not 12?

Let's start w/ Column t tests:

Columns:

1-2	$p = 0.20$	The tells us, that
1-3	$p = 0.13$	w/respect to detergents,
1-4	$p = 0.11$	there is a difference
2-3	$p = 0.55$	occurring between
2-4	$p = 0.42$	detergents 1 & 4
3-4	$p = 0.71$	as we suspected.

Now, to test row interaction, you need to enter the data on a transposed matrix.

Row interaction tests:

1-2 $p = 0.89$
1-3 $p = 0.37$
2-3 $p = 0.41$

These gives us exactly the results that we expected. There is no significant difference w/ respect to the water temperatures used, only two of the four detergents significant @ the 89% level.

Now this gives us 9 out of 12 combinations. They are all occurring when $i \neq j$.
Now how do we handle the case when $i = j$

$i=j=1$	$i=j=2$	$i=j=3$
37 37	45 56	30 72
56 45	22 22	34 40
72 30	40 34	28 28
22	27	37
$p = 0.15$	$p = 0.93$	$p = 0.25$

Thus therefore addresses 12 combinations, the max that should be possible.

I think that we have developed a method to explore the interactions between each factor combination. Our box plot showed up the labels/results.

The box-plot shown that the means vary the most between Factor Detergent Columns 1-4. ($p = 0.11$)
The matches our result.

The next two means that vary diffr the most are Columns 1-3 ($p = 0.13$)
The also matches our t test results on Columns.

Now, to look @ the results w.r.t temperature graphically, we need to form box plots for the transposed matrix since the graph can only portray column box plots, not row box plots.

None of those are close to sig, so there is no real advantage in doing so.

Our next most significant results is when $i = j = 1$ (ie, Detergent 1 Combined w/ Low temperature). Let's form that box-plot.

List 1	List 2
37	37
56	45
42	30
	22

And, most certainly this box plot shows us visually the greatest difference

The ANOVA method is useful because it gives us a quick analysis if there is some kind of important difference resulting from the factors or their interaction.

The multiple comparisons ARE REQUIRED to isolate which combination is likely causing the difference.

Unless the matrix is quite large, it is not very difficult to re-enter a transposed matrix in the i=j case to explore the i*j combinations.

The box plots are useful for a visual perspective on the situation, however the t tests pare down a 'p' value for each combination.

All in all, this has been a most fruitful exploration.

Our next topic is apparently Chi Square Testing, ie independence of factors.

Aug 21 2019 - Swan Lake, MT

Exactly one month later. The camp approximately 30 miles SE of Kalispell MT should offer the quiet & concentration needed for some studies here.

There is a documentary film crew coming here in a day or two who some work will be demonstrated & some issues discussed.

Four primary laboratory methods are available here in the field on this trip:

1. Microscopy
2. Near Infrared Spectroscopy (NIR)
3. Titration
4. Electrochemistry

as well as some basic qualitative chemical methods.

Microscopy alone is sufficient to classify & consume most any items available here over the next couple of weeks.

Microscopy, at the right time, is the most important & valuable of methods to use. This is such a time.

A variety of samples of interest have now been received - one to come from Italy.

The heightened interest in sending samples to me likely stems from recent papers written, such as "The Evidence is Evident". That paper records an extraordinary sample that shows the filament growth directly from a toenail. It is extraordinary.

I likely have a half dozen + samples now of interest to view.

1. The first will be my earwax. I will be writing on health and therapeutic strategies again in the near future as some important testing of methods has been underway. A chronic ear infection issue is one of the topics @ hand.

Let's work out magnification Capabilities first.

24 divisions are available w 40x eyepiece.
Each division is 0.01 mm = 10 um
So we are covering 240 microns @ 40x with a screen scale of 30°.

Our current magnification is:

$\frac{240\ \mu m}{12.5\ cm} \Rightarrow$ Magnification of 520.8

Now since since we are at a screen scale of 30%

our actual magnification is @ $\frac{100}{30}$ (520.0)

$= \underline{1736\times}$

Now if we are able to get the last objective we have available

$\frac{100\times}{40\times}$ (eyepiece) $\cdot\ 1736\times \Rightarrow \underline{4340\times}$

which we can round off @ $\underline{4500\times}$

So for various objectives we have

Objective	Magnification Ratio
100×	~4500
40×	~1750
10×	~450×
4×	~175×

Now the examination of the chronic liquid (right) ear wax begins @ 175x.

@ 175x:

The amount of detail that can be ascertained is unremarkable. We primarily have a granular structure visible @ the stage. A series of filaments can also be observed but not be too large to assess their nature @ the low magnification. They could be easily be hairs, although one is red-colored.

@ 450x:

Here we see slightly increased resolution of the granular matrix. We are dealing with a large quantity of small spherical objects; they may well be the Coccus form.

We also have two forms of filamentous visible. One is a clear (hair) and the other is not. That which is not has the granular twisted ribbon structure.

Now let's proceed to 1750x.
Our most likely optimum magnification achievable.

OK. 1750x is sufficient to prove the case.

Clearly the smallest identifiable feature in the coccus form.

The size of this is approx 2 mm @ 100x

Therefore the size extended is $\frac{2\text{mm}}{1750\text{x}}$ =

$= .001\text{ mm} \cong 1\text{ micron}$

Exactly what we expect w/ the equipment that is available.

Higher end equipment will resolve the diameter to the sub-micron level.

In addition the twisted flattened filament form exist occasionally throughout the sample. This further confirms the fact that the Chlorella ear infection is based upon a CDB infection.

In addition, the CDB can be seen to be aligning themselves frequently in a collinear fashion. This is known to be the early stage of filament production.

Refer to the "Growth Progression" research paper that demonstrates this fact.

The next sample being reviewed is a rather thick & plasticized sputum sample that appeared approximately a month ago. It has been preserved in a dried state and is now re-hydrated.

First magnification @ 450x.
With only introductory examination, the first remarkable point of interest is the existence of a bright bold flattened twisted filament of internal structure already visible. It appears to be of Clostridia form.

Two separate filament selection appear, along w/ constellation of neutral color.

The sputum sample is somewhat indeterminate W.r.t. repeatable, identifiable CDB existence. Only the presence of the flattened, twisted, color filaments is to be recorded here.

Aug 22 2019 Filaments in Teeth - Proven.

The next analysis was that of two teeth received from Canada.

The claim was that filament structures are emanating from the teeth.

This claim has been verified as being true.

All examination has been done under low power w/ the USB microscope.

The filaments are difficult to observe but are visible. Examples were found on both teeth examined, however, one tooth has more than the other.

The filaments are frequently embedded w/in the tooth. They are also seen to project outward about a mm or so from the tooth exterior on occasion.

The submission of the sample was based upon publication of the paper

"The Evidence is Evident"

that shows the filament growth from a toenail.

The observation of filament growth from teeth is a great deal but is extraordinary. It shows the insidious nature and depth of infection by the organism w/in the body.

The individual has lost their teeth from the Morgellons condition @ at a early age.

The same happened to me. I lost all teeth, (upper) also known to be from the cause. I suffered greatly for more than a decade before I was allowed to have my teeth removed.

I am seeking the possibility of examining additional teeth from the same individual.

In addition, I have saved my own teeth removed years ago and it begs the question as to whether my teeth will show the same result. I have not examined them to the detail required to establish the validity of the claim that has been made here, and that is now known to be true.

The sending of the teeth and would kindly exercise very good observational & microscopy skills to discover the activation.

Skin Crystal Examination

The next sample examined is that of a crystal like material that has emerged from the skin. The sample came from the same individual that provided the hair w/ the filament network that is the subject of the "The Evidence is Evident".

This too, shows itself to be a groundbreaking sample in terms of its revelations.

By all appearance, the sample appears to be similar to a fleck of "aluminum foil".

But it is not "aluminum foil by any means.

Because of its general opaqueness, it is a difficult subject for microscopy.

Fortunately a segment of the crystal is translucent and can be examined.

The discovery is that the crystal has exactly the sub-filament 9 "CDB structure" * that is known to be Characteristic of the infamous EPA "Environmental Filament" of the first studies circa 2000.

Many crystals are reported anecdotally in crystal formation in the skin. I have now witnessed a total of three such samples. Two of them are hexagonal and the particular one I'm using is irregular in shape.

This is the first sample w/ sufficient translucence in the crystal to allow light phase microscopic examination, in the case ~ 1750 X.

The discovery here also leads to the conclusion that the CDB does not form only the filament network (& beyond, for example) but that it can and does also produce

Crystalline Structures.

* This is another important discovery in the process.

Skin Crystal Examination II

The last observation set is equally important and profound.

The observation set consists of a set of four reflective hexagonal crystals (leafs) approx 1 mm in diameter. This is the classic form that is reported.

The crystals are opaque, and therefore very difficult microscope subjects @ higher power.

With much patience & observation, I was able to work w/ one of the crystals through higher power (~1000x). It was observed that one of the crystals contained a more translucent edge & work was focused on that crystal.

The observation conclusion is that, once again, the smallest identifiable structural unit within the crystal structure is the CDB.

The confirmation from two independent crystal observations (the crystals have been removed from the skin)

that the origin of formation, from all available information, is the CDB.

The [illegible] substantiate the claim that the COB are now known to be @ the origin of both the judgment networks AND the crystalline structures that are repeatedly identified and observed.

Aug 23 2019

I have a slide of the original EPA "Environmental Filament" available. In a sense this started it all, and dates back to 1999.

It will be photographed as a reference for the current Microscopy session, Taylor 20+ years now.

Excellent microphotographs taken.
Achieved up to 4500X. CDB are visible.

KRT skin filaments (surround hair shaft) photographed up to 4500X. CDB clearly visible.

Sep 01 2019

There are many things of importance to discuss and reveal. Only an outline can be presented now. Topics include:

1. A major blood change discovery. The one is profound. Essentially it mimics the observations of the

"Complex Observations, Unknown Consequences"

The immediate need is for more data and a variety of blood samples. At this point, I can only say that a stage may be being set for the presentation of a paper titled:

"The Transformation of A Species"

This is a heavy duty topic. I will reveal more when and if I can.

2. Many health trails are active, under evaluation & subject to experimental protocols.

The primary locations affected are

1. A Chronic & significant ear infection, the liquid wax debris has been proven (microscopically) to consist of the CDB along w/ occasional Clavus filament presence.

2. Significant attacks upon important nerve centers w/in the body (nerve & joint).

Some of these include:

1. The right shoulder (chronic for many years but the majority of the pain has now been relieved)

2. The knees, stronger w/ the right knee. Most of the pain has also been relieved

3.

3. The A knuckle on the left hand. The pain from the apparent have been relieved also.

4. A major spasm-attack on the lower back. The event was entirely debilitating & severe. The also seems to have largely returned to normal.

5. The base of the left shoulder blade on the back. The location is very very difficult to access and cannot be reached by hand.

The location remains a primary problem, and it affects the nerves throughout the lower left arm and hand.

There is focus area & therapy for pain reduction.

6. There is also a chronic low level upper right lung respiratory issue that has been mentioned previously.

7. The Thyroid - node issue - Potentially serious.

Three primary mitigating strategies are in place and in use:

1. Ultrasound
2. TENS (Electrical nerve stimulation
3. Methyl Salicylate balm (~ 15% concentration)

What is unique about the nerve center maladies is that they move and relocate with-in the body, often quite rapidly (eg < 24 hrs).

This is why arthritis is not a serious candidate but the CDB networks that are created w/in the body ARE.

Ultrasound is known to be highly disruptive to the microbial ensconcements, but at this time does not eliminate the presence. Simply put, the pain will move & relocate upon the body to a new location.

The shows the effectiveness of the current back-lower shoulder blade location which is highly difficult to access.

Clever use of the ultrasound appear to be the most promising strategy. TENS is difficult to apply there.

Today I am working on the thyroid question using Near Infrared Spectroscopy (NIR).

It is known from professional ultrasound that I do have a large nodule in the right thyroid.

I have decided to investigate this w/ NIR today.

The signature difference w/ NIR between the left thyroid (presumed as "normal" and acting as a reference) and the right thyroid (where the nodule is known to exist)

is quite profound.

Significant absorbance peaks are showing up in the right throat - thyroid region at:

~ 1440 nm (strong) OH or CH (sucrose, starch)

~ 1200 nm (medium) CH - fat detection

~ 920 nm (weak) CH - oil

The analysis of this differential NIR spectrum of the right thyroid region indicates that the composition of the nodule is primarily that of fat or oils.

This seems to be an entirely consistent set of conclusions known thus far.

The ultrasound classified it as a cyst or a nodule 2.5 cm in size. Nodules are apparently of liquid state and are usually benign.

A fat, especially oil-like composition is entirely consistent with this. If it were to have been NH bonds indicating possible protein composition, this could clearly be interpreted to be a cancerous or tumorous proportion.

We must act on the assumption that we need to, with the available information, determine if ultrasound might be effective in breaking up such a fat ball or oil based nodule.

Caution is anticipated here. A sudden discharge of concentrated fat globule in a small & vital organ could be very dangerous & high risk.

Sep 02 2019

Let's talk about the ear ~~door~~ situation a little more.

It is established that a chronic right ear situation exists. Highly liquid wax is produced on a continuing basis. It is now known (as suspected) that the wax / fluid is dominated in composition by the COB and occasional classic structure filaments. Some normal hairs were also observed in the microscope session.

It is also known that Ultrasound is having a definite effect upon this situation, gradually to the betterment without question.

This ear situation has gone on for several years but has only been chronic for approximately the last six months.

It has been common (on average up to 1 in 3 days) that my ear is plugged w/ the wax and often I do not feel well in accompaniment w/ that.

One of the more recent ultrasound sessions is of particular interest.

During a more sustained session on the ear, also preceded by a debilitating muscle spasm in the back, I decided to use the ultrasound @ the highest setting. Time applied was up to approx 30 min.

There was more heat generated in the application of the higher strength ultrasound, and I was aware that certain unknowns & potential risks were at play.

What occurred subsequently over the next two days was somewhat extraordinary.

The first sensation, rather prompt, was that of the heated area (most likely of a fluid nature) seemed to "open up" and then dispersed slowly across the back of the neck over the next several hours. It was apparent that something different had occurred during the session.

* I subsequently became at least 95% deaf IN BOTH EARS over the next two days.

It was, at the time, completely unknown if the deafness was permanent or temporary.

It was quite clear that the fluid redistribution in the back of the neck was responsible for the deafness.

After a couple of days, hearing slowly returned to the "normal" state.

Since that time, the impact of the chronic ear infection seems to have lessened, but it is by no means gone.

My ear still plugs itself up on many days, especially in the waking hours. What is different is that I usually can open it up for hearing each day by a massage to the right ear.

This remains the current state of affairs.

My current plans are to begin applying the ultrasound on a more sustained basis (if possible - it does require ~½ hour per location) at the following locations:

1. Continuing w/ the right ear

2. The lower left shoulder blade - upper back - a major nerve - pain center in here)

3. New, and at a level of experimentation & unknown, will the right thyroid area. This will need to be conducted gently, especially @ first, to sense out any unknown reactions that may take place.

Now let's mention the blood charge a little further.

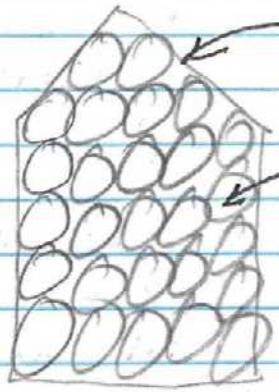
I intend to examine some alternate blood samples from other individuals for comparison, as this is the first requirement.

Only one person of the nature has taken place thus far, and it is only circumstance that allowed me to see what I have seen.

It is a very prerequisite item to be familiar w/ the microphotographs of the urine sample posted on the paper,

"Complex Observations, Unknown Consequences"

You will see the following:



The lay interpretation of what is seen by most people would be that of a

"Computer Chip".

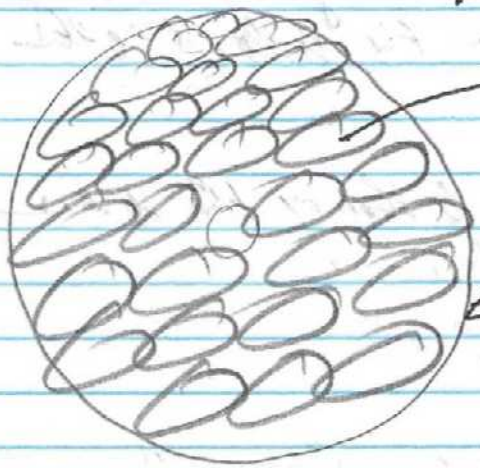
My interpretation @ this time is:

"Potential Biological Circuitry"

As we see that both interpretations are closely related to one another.

Further study will be taking place but it is necessary and appropriate to convey what has been seen in my blood.

Essentially every blood cell seems to show these development. It is of the form:



Individual CDB

Blood Cell IS INTACT w/ structural & membrane integrity

It must be recalled that earlier entrance of the CDB into blood-cell samples resulted in the paper titled:

"A Mechanism of Blood Damage"

The damage to the cells was extreme during the time of that report. I considered myself fortunate to be alive at the end, for the damage was severe.

What has taken place here (permanence of the Condition is unknown at the time) appears to be extra ordinary as the cellular membrane does

FULLY INTACT

and an organized assemblage of the CDB has taken place w/in the blood cells (my blood)

in a manner similar to that of the urine sample first reported on.

A prospective title of the
"A Transformation of a Species"

As I mentioned, may be loonery.

It is unknown @ the time of my extraction as unique. Other than the health histories described I have no reason to assign ill health to me.

More samples & observations are required to gauge the extent of what has occurred here.

* All appears to be quite extraordinary.