CARNICOM INSTITUTE LEGACY PROJECT

A Release of Internal Original Research Documents

Authored
by
Clifford E Carnicom
President, Carnicom Institute

Laboratory Notes Series: Volume 23
Jun 2018 – Dec 2018

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.

PERFECT EDGE ® MICRO PERFORATED FOR CLEAN TEAR-OUTS

5 SUBJECT NOTEBOOK

180 SHEETS

MADE IN USA

College Ruled
10.5 IN x 8 IN

LAB NOTEBOOK

VOL XXIII

Norcom Inc. Griffin, GA 30224
Item#77186

Volume 23

Jun 2018 – Dec 2018

Jun 29 2018 Page 1

Still on Abajo Mtn.

Let's continue w/ some of the moderate priority spectra.

What we have is a skin foliation sample from an individual that exhibits classic skin morphellone condition on the legs. Samples were photographed under the microscope; classic bundled & colored filaments were visible in the sample.

Although the spectra information is fairly unusual, the spectra should be recategorized as high priority, which will soon be evident.

This is a microwave NaOH digested sample.

We have absorption peaks @:

3371	2061	1631	1283	~650
✓	✓	✓		✓

First question always - do we have a carbonyl?
yes: Carbonyls (1600-1820). We have 1631.

We have the amide again. Required:

~ 1640-1610	C=O stretch
3500-3100	N-H stretch
1640-1550	N-H bending

We also know that the we have the Isothiocyanate @ 2061
Understand we expect the amide w/ an ester, but not the Isothiocyanate.

The strong peak @ ~ 650 brings us the alkyl halide again.

The spectrum is prior to recent maintenance & calibration of the IR instrument, so we are unable to establish the alkyl halide w/ full certainty again, but all appearance will again be with C-Br.

This leaves the weaker absorption @ 1283 cm^{-1} remaining.

We do have amide (III.) by Parker from (1305-1200).

This is the most direct assignment that remains consistent.

They are the summary is:

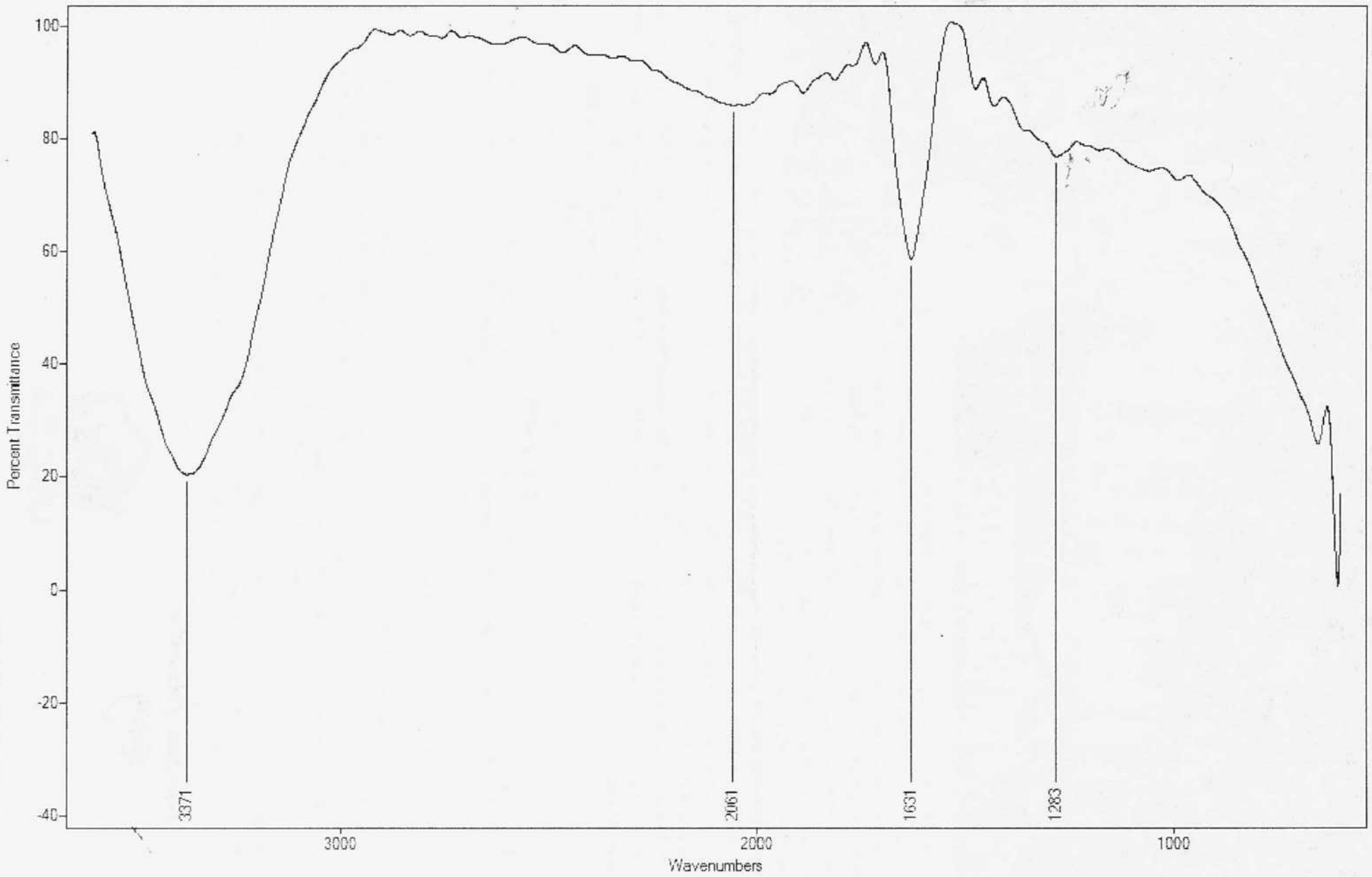
1. Amide (ie protein)
2. Isothiocyanate
3. Alkyl halide

A pattern is quite apparent by now.

Aber Foliator Sample – Mazellon's symptoms exhibited.

Skin Foliation NaOH Microwave ATR Aug 28 2017 - 01.spc: Synthetic spectrum by averaging. See audit trail for details.

3A



printed on 06/11/2018 09:44:00

Our next sample that appears in the moderate priority list is that of CDB headspace analysis.

The case is headspace, not pyrolysis. Presumably the heat applied is relatively modest. Gas cell analysis would have been used here. The noise level is high & therefore the spectrum is apparently w/a weak signal. Notice that no thiocyanates are not present and that the headspace result may vary considerably from the pyrolysis examination.

1st, do we have a carbonyl? 1707 cm^{-1} says yes. (1820-1640).

This is again in the range of amides.
Carboxylic acids are @ 1710 so we will keep this in mind.
The acid does not work as it usually overlaps the C-H region (that is an important and characteristic) and in addition, the absorption is very broad (also an important characteristic).

By Parker, amides are
1610-1640 C=O stretch
3500-3100 N-H stretch
1640-1550 N-H bend

The carbonyl forces us to the amide vs amine side. If we did not have the carbonyl it is then appropriate to consider the amine.

However, we notice we are a bit high w/ 1707 cm^{-1} relative to ~~Parker's~~ Pavia's boundaries @ 1670 & 1640.

Parker gives us the secondary amide up to 1700. (1700-1670).

Notice that we have some additional shoulders above 1707 (amine near 1750).
Keep an eye for ester tied in w/ this region.

Notice indeed that both Pavia & Parker raise the ester prospect.
Pavia has the ester @ 1735.
Parker has the ester @ 1745-1735.

? [The suggests our amide might be joined w/ or influenced by an ester.]

We have another strong peak @ 1043.
Our strongest candidate is sulfoxide >S=O
1050-1020 Parker

What does COB Dyrolyser show?
We do indeed have previous peaks of 1018 & 1025 that have been assigned to the sulfate group.
The assignment holds w/ a slight modification to sulfoxide.

We notice the strong absorption again ~650.

The again belongs in the alkyl halide.

With the exception of lack of isothiocyanate presence (recall that we are dealing w/ a headspace vs a pyrolysis sample) we seem to have amazing corroboration once again.

Our summary for the CDB headspace is:

1. Amide (i.e., protein)
2. Ester likely
3. Sulfoxide
4. Alkyl halide

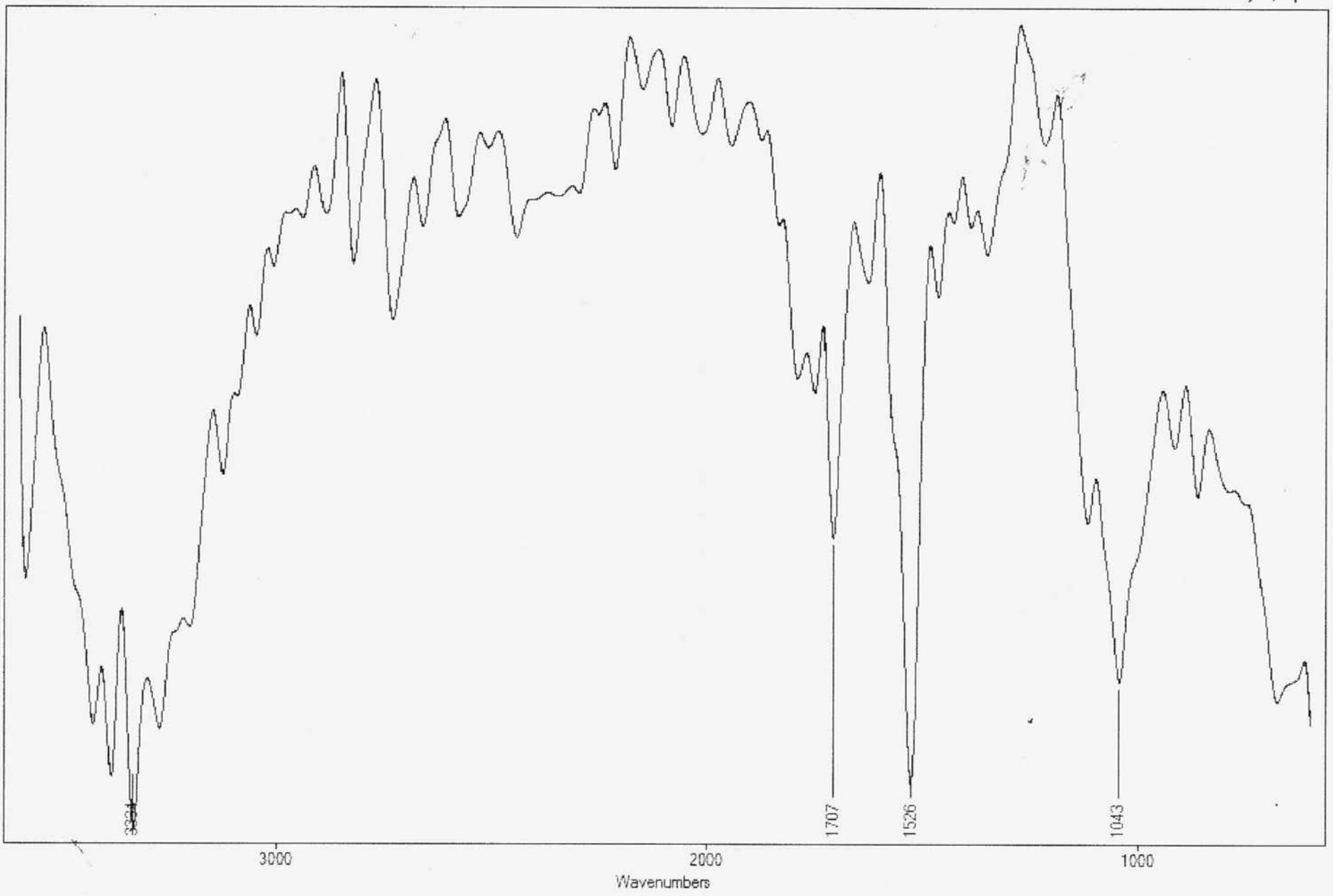
This concludes the moderate priority spectra.

CDB Headspace Sample

Moderate

CDB Gas Analysis - Headspace Aug 09 2017 - 01.spc: 08/09/2017 14:40:20 title

7A



printed on 06/12/2018 20:57:34

Next I would like to consider the spectrum of collected materials from a shower sample. Protozoa were also observed w/in the liquid sample, along w/ the solids present. The solids were of two forms, floating & settled, and they have been photographed under the scope.

The spectra of the ~~sediment~~ settled material. The spectrum appears to be rather complex in the fingerprint region. We also have the spectrum of the floating material, which appears to be less complex.

The sample is of interest because of
1. The volume of settled material
2. The claim of washing off from the shower, presumably w/ dirt soap. (confirm this)
3. The floating material appears to be of a polymer form, similar to what has been observed in various uncontrolled cultures, especially w/ soap present.
4. Additional & separate samples from the individual presented classic filament structures.

We start w/ the settled material spectrum:

Do we have a carbonyl? yes, @ 1645. (1020-1600).

Once again, we have the amide absorption @ 3318 cm^{-1}.
Expectations:

1670-1640	C=O stretch	all satisfied for amide prelude.
3500-3100	N-H stretch	
1640-1550	N-H bend	

—

We clearly have the hydrocarbons here w/ strong absorption @ 2926 & 2855.
from Avram, this is symmetric & asymmetric stretch of CH_2. (Avram p130-131)

—

We have extremely strong absorption @ 1084 cm^{-1} w/ a shoulder @ 1029 cm^{-1}.

Our strongest candidates appear to be:

str 1030-1090 P-O-C phosphoric ester (very strong)
str 1020-1090 Si-O-C trimethylsilyl (very strong)

We seem to be a bit high for sulfur compounds here.

From Pavia, an ester requires 1735.
We do not have it.

This shifts the focus towards Si-O-C w/ reconsideration of sulfur compounds that can range from 1020-1058. Both sulfur & Si compounds are in range here.

As we continue here, we must also consider that we have a plural compound here, especially due to the complexity of the fingerprint region.

Thus far:
1. Amide (protein)
2. CH_2 hydrocarbon.
3. Sulfur and/or silica consideration

1525 can be considered next.

This corresponds well the amide.

Parker
- 1510-1515 Amide II (solid) secondary amide
- 1550-1510 secondary amide (solution)

Assignment of 1525 goes to the amide.

Our next peak is @ 1412.

This also assigns to the amide by Parker.

Parker
- 1418-1400 primary amide
- ~1410 aliphatic amide

Next we can go to 800 cm^{-1}:
Parker has an amine salt here.

Parker
- ~800 NH_3^+ amine salt
- ~800 NH_2^+ amine salt

This says that we may have both amines and amides — this suggests multiple components within.

Our last peak of consideration is 1266 cm⁻¹. It will also assign to the amide:
Parker 1305-1200 N-H secondary amide (III) (med)

We go no further @ this time.
We suspect a possible multiple compound.

1. Amides
2. Amines (Amine salt)
3. Hydrocarbons CH_2
4. Si or S compound. Organo sulfur

More information on the specifics of collection are desired. Is soap involved?

The person can be contacted.

This concludes spectral interpretation for the moment.

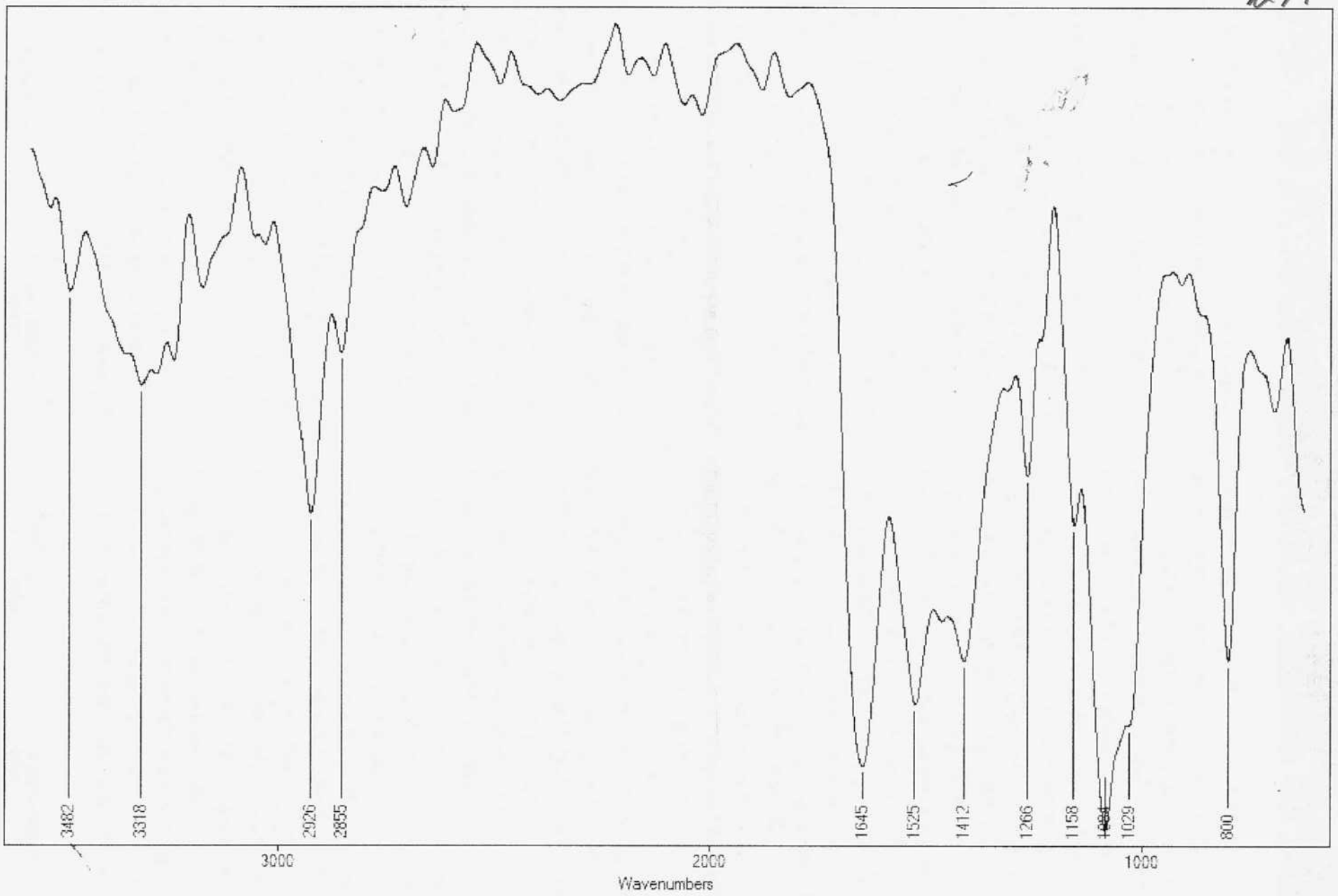
The floating sample can be considered, however.

Shower Sample: Settled Material

[redacted] Shower Sample - Sediment Material - ATR Jun 07 2018 - 02.spc: 06/07/2018 15:00:07 title

Moderate

12A



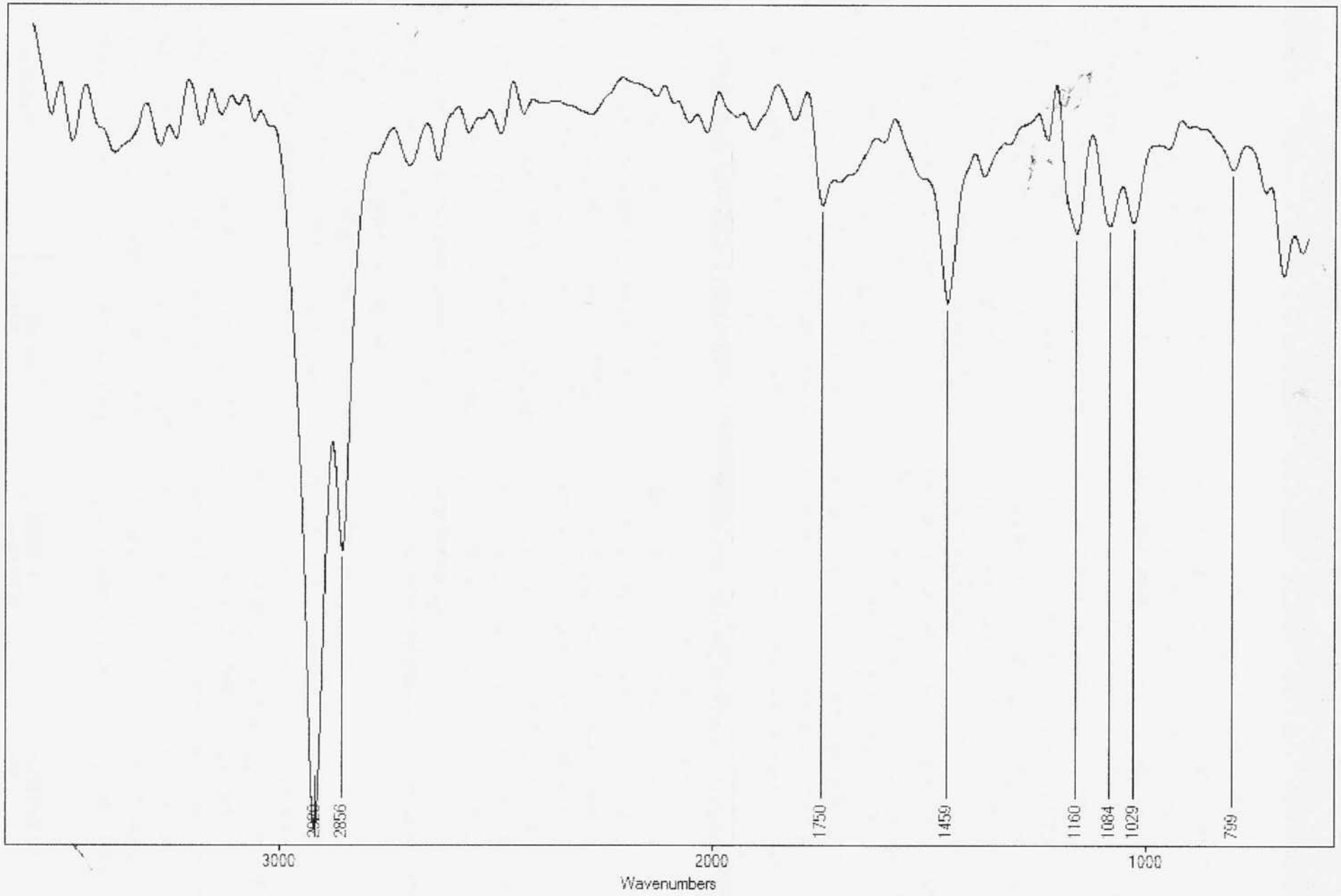
eFTIR

printed on 06/07/2018 15:15:57

Shower Sample: Floating Materials

[redacted] - Shower Sample - Floating Material - ATR Jun 07 2018 - 01.spc: 06/07/2018 14:47:13 title

13A



printed on 06/07/2018 15:13:53

Let's continue w/ the floating shower sample material, strong on hydrocarbons.

First, do we have a carbonyl? Yes, 1750. (1820-1660).

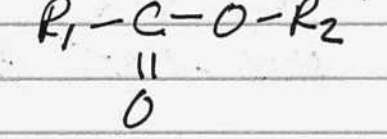
From Pavia:
1760: Anhydride (Band 2)
1810: Anhydride (Band 1)
1735: Esters

Our bias in the spectrum is below 1750. This directs somewhat toward the ester but it is required to learn more of the anhydride, and the distinctions between Band I & II.

Anhydrides, per ~~Past~~ Pavia p 420 state both absorptions @ 1810 & 1760 are required for anhydride.

We are therefore directed toward the ester. Let us see what may cause a shift in the ester toward 1750.

First off, the ester is:



From Avram, it is very easy to have the freq shifted to ~ 1750 cm⁻¹.

The addition of hydrocarbons (most definitely present), halogens, etc easily bring about this shift.

There are esters w/ hydrocarbons to assigned to 1750 cm^{-1} absorption. (conjugation in R' moves absorption to the left - Pavia).

Our next most significant absorption take place @ 1459 cm^{-1}.

from Parker:

~ 1468 Alkane $-CH_2-$

~ 1460 $-CH_3$

Let's look @ our hydrocarbons before going further:

2920 & 2850

from Avram, these is CH_2. (Avram p131)

from Avram we would expect to see CH_3 @ 2962 & 2872 We do not. Therefore we at the time, restrict our assignment to the CH_2 group.

Our next peak of significance is 1160 cm^{-1}.

Parker does seem to focus on C-H bonds here

1170 - 1140 $(CH_3)_2C-$ [isopropyl (?) added]

1175 - 1125 C-H unsubstituted phenyl

What is phenyl? Benzene minus a hydrogen replaced by another functional group or Compound.

The phenyl group does not match.

The $(CH_3)_2C<$ does, and is our second indication of now having both CH_2 & CH_3 present. The presence of these HC's also does seem to be exactly what can shift the center freq of 1735 to 1750.

Two ~~The~~ additional peaks of interest remain:
1160 1084 1029

1084 has been discussed on the settled material analyses.

Si-O-C
or Sulfur Compounds are the assignment Candidates.

This particular absorption shows a linkage between the floating & settled compound structures.

And lastly, the peak @ 1029 is also common to the settled material which we have assigned to the sulfoxide group
$>S=O$

from Parker
1050 - 1020 S=O stretch $>S=O$ sulfoxide

In summary for the floating sample:

1. Ester
2. Hydrocarbons attached CH_2, CH_3
3. Si-O-C or Sulfur groups
4. Sulfoxide

In light of #4, simplification supports the existence of organo-sulfur compounds over the Si-O-C candidate

I will therefore reduce the summary to: (floating sample)

1. Ester
2. Hydrocarbons attached
3. Organo sulfur compounds

Primary Distinction

vs the ft settled materials:

1. Amides
2. Amines (amine salt)
3. Hydrocarbons
4. Organo sulfur compounds

Shared

These findings increase the interest level in the specific collection methods for the sample and whether or not it can be repeated. Recommend contact of the individual.

** The Paper to come forth shall be entitled:

A Point of Reckoning IV
or
Thiocyanates:
Where Else Art Thou?

There are four papers to be written:

1. Summary of IR chemical signatures of a broad array of sample types

2. Blue protein

3. "Confronting Geometry" - "The CBD form"

4. The distribution of methiocyanates across a broad array of samples

—

and the epitath shall be:

Clifford "E" Carnicom
Born Clifford Bruce Stewart Jan 19 1953
We get to save the death for now

[redacted]

Researcher for the Benefit of Humanity

(Note - Sep 01 2020: I am not rushing anything here. In fact, I have put in several requests along the line for extensions, which have already been granted. Needless to say, I am grateful... CEC)

Jul 31 2018

A little regrouping here. I see that Conley (Infrared Spectroscopy, 1966) is actually an excellent additional reference, and that it should be consulted in the process. Especially if there is some uncertainty involved or a lack of corroboration at the level desired.

Two cases come to mind. We have no real evidence of alkyl halides beyond the conventional IR presentations in books on organic absorption in the general range of 900-600 cm^{-1}. What are the alternatives?

In addition, ambiguity creeps into play with the phosphorus, silica + sulfur absorption regions. The absorption of inorganics and "organics w/ heteroatoms" also falls outside of the normal + conventional IR textbook presentations.

These are both critical subject areas, and I am involved w/ them in my work here. The point is that

* Conley is covering these topics extremely well.

Conley has several important sections:

Page 91 Hydrocarbons (traditional)
Page 110 Major Functional groups (traditional)
Page 171 Full Organic Correlation Chart (traditional)
Page 176 ORGANICS w/HETEROATOMS (S, P, Si, etc) Metals
Page 184 INORGANICS

Fantastic additions

These are fantastic additions.

These two additional sections in Conley are to be considered very carefully.

Any time that we are involved w/ the
sulfur
phosphorus
silica
metals
} questions we must consult Conley carefully here

Any time that we suspect that we are dealing w/ inorganics we must consult Conley carefully here.

Any time the halide region becomes active we must consult Conley carefully, esp if there is no known additional evidence to support the halide conclusion.

So the means of we go.

Final requirement is to review our spectra assessments and identify any such points of possible confusion or misdirection.

It will help to summarize exactly what spectra we have worked on here.

Our set includes - (It will be taken to go in reverse)

1. Shower sample floating (Jun 29)
2. Shower sample settled (Jun 29)
3. CDB Headspace sample (Jun 29)
4. Skin foliation sample (Jun 29)
5. CDB Viscous Protein (Jun 28)
6. Embedded Skin Crystal (Jun 20)
7. CDB Pyrolysis (Jun 27)
8. HEPA filter non-polar (Jun 19 summary)
9. HEPA filter polar
10. Rainfall extract non-polar
11. Rainfall extract polar
12. CDB Viscous Protein (1st gen)
13. The environmental filament.

This is indeed quite a set.
It will undoubtedly be helpful to list the findings from all 13 samples.
This will require a full page spread.

The IR Findings thus far: (Tentative)

1. Shower Sample Floating

1 1. Ester
1 2. Hydrocarbons attached
1 3. Organo-sulfur compounds

4. Skin Exfoliation Sample

3 1. Amide
1 2. Isothiocyanate
3 3. Alkyl halide

2. Shower Sample Settled

1 1. Amides
1 2. Amines (amine salts)
6 3. Hydrocarbons
2 4. Organo Sulfur Compounds

5. COB Viscous Protein

4 1. Amide
1 2. Iron
5 3. Sulfur
4 4. Alkyl halide

(Iron-sulfur protein)

3. COB Headspace

2 1. Amides
5 2. Ester likely
3 3. Sulfoxide
1 4. Alkyl Halide

6. 12. COB Viscous Protein (1st pass)

~~5~~ 1. Amides
2 2. Iron (Fe^{+2})
~~6~~ 3. Sulfates (SO_4)
4. Ferredoxin Protein

7. COB Pyrolysis

2 1. Ester
4 2. Sulfonic Ester
2 3 Isothiocyanate
2 4. Alkyl Halide

6. Embedded Skin Crystal

6 1. Amide
5 2. Cyanogen derivative
1,3 3. Isothiocyanate
5 4. Alkyl halide

10. Rainfall Extract - non polar

4 1. Hydrocarbons (aliphatic)
3 2. Hydrocarbons (cyclic)
3 3. Ester
10 (Sulfate ester emphasized)

8. HEPA filter - non polar

2 1. Alkanes
12. Cyclic hydrocarbons
3. Aldehyde
8 4. Thiocarbonyl

11. Rainfall Extract - polar

5 1. Alkanes
2 2. Amines
3. Ether
11 4. Sulfates
4 5. SDBS best match is sulfate ester

9. HEPA filter - polar

3 1. Alkanes
2 2 Cyclic Hydrocarbons
3. Aldehyde
9 4. Thioaldehyde

13. % Environmental Filament

7 1. Amides
13,4 2. Isothiocyanate
3. Nitro Group
124. Sulfur Group.
3. Ferricyanide

Emphasis upon Tentative.
I will be reviewing the "alkyl halide" situation

Word Count:

- ✓ ~~Ester~~ 4 5
- ✓ Aliphatic Hyrocarbons ~~5~~ 6
- ✓ Sulfur/Related Compounds ~~12~~ 12
- ✓ Amides ~~4~~ 6
- ✓ Amines 2
- ✓ Isothiocyanate, ~~4~~ Cyanide derivatives 5
  Iron ~~X~~ 1 6
- ✓ Cyclic Hydrocarbons 3
- ✓ ~~Alkyl Halide~~ 5 Ferricyanide
- ✓ Aldehyde 2
  Nitro Group 1
  Ether 1

Ranked:

		Normalized: %
1. Sulfur/Related Compounds	12	100
2. Amides	6	50
3. Aliphatic Hydrocarbons	6	50
4. Ester	5	42
5. Thiocyanate, Cyanide Derivatives	5	42
6. ~~"Alkyl Halides"(?)~~	~~5~~ 6	~~42~~ 50%
7. Cyclic Hydrocarbons	3	25
8. Amines	2	17
9. Aldehyde	2	17
10. Nitro Group	1	8
11. Ether	1	8
12. Iron	~~4~~ 6	~~8~~ 50%

See Notes 00730

Ferricyanide

Ok, this gives us our first overview of high priority functional groups across a wide variety of sample types.

Our highest interest is with:

1. Sulfur related compounds
2. Alkenes (Proteins) → Iron
3. Hydrocarbons (expected) → Ferricyanide
4. ~~Esters~~
5. Thiocyanates & Cyanide derivatives
6. ~~The purported "alkyl halide"~~
7. Cyclic hydrocarbons
8. Thioaldehydes as to be obtained

Now, we have a major concern based upon the "alkyl halide" assignment. This was based upon a conventional organic IR interpretation. This may be a very faulty approach, which is revealed with the examination by Conley.

Conley has a very important section (amongst others) on inorganic absorbtions - This chart is on p 192-193.

The sulfur and iron absorptions are of great interest to me because of their known presence and distribution w/in the ODB and the produced proteins.

For iron, a very intriguing single absorption is given from ~650 to 600 cm^{-1}, and it is for

$Fe(CN)_6^{-4}$. this is ferricyanide.

This is actually of low toxicity, but irritant to eyes and skin.

However, there are some conditions to the statement, as well as exceptions it seems.

It can be toxic under $6H^+ + [Fe(CN)_6]^{3-} \rightarrow 6HCN + Fe$

1. strong acid conditions (HCN gas is released)
2. photo-decomposition
   (ie exposure to light, very toxic, 1950 paper on fish mortality)

$K_3[Fe(CN)_6]$ is potassium ferricyanide

It is a deep red solid soluble in H_2O.

Another source says it is non toxic in alkaline solution.

It is somehow related to Prussian blue.

Ferric ferro cyanide $Fe_4[Fe(CN)_6]_3$ is Prussian Blue

" Ferro and ferricyanides, as oxidizing agents, would be expected to induce methemoglobinemia. "

Recollections on early studies anyone?

" Cyanide complexed to metallic salts is generally considered weakly toxic since the tight binding between the metal and the cyanid group prevents cyanide release "

It appears we may be on to it here.

Okay, another discussion is in order.
Microscopy of skin flakes has recently (within the last few days, revealed a most important finding. The finding relate to the observation of numerous circular blue tinged structures that have shown up within both culture & biological samples.

It is now understood what these " blue circles " are.

The observation is this. If a skin flake is placed under the microscope, no colored chemical reaction take place. The presence of the colored filaments, however, has been observed and recorded (multiple filaments) even within a 2mm x 2mm skin sample. This is the first unusual finding, and it has been repeated 4/4 in 2 of 2 attempts. No outward signs of illness, etc lesions of any kind appear.

Here is what happens:

If you now place a single drop of water on the sample, i.e., make a wet slide of the skin flake sample, the "blue spheres" will be produced.

The "blue spheres" are air bubbles that are being formed as a result of a chemical reaction that between the water and the skin.

At the boundary, or interface, of the air bubble and water, a rich blue pigment, or color, will be produced. The pigment will then dye the surrounding cells.

This is the identical feature and reaction that has been repeatedly observed in both cultures and histological samples.

* What is different is that the reaction can now be produced with ease, at will and under controlled conditions.

It will not be a total surprise if this reaction does indeed involve Prussian Blue.

All of this take place and can be recorded under the microscope within the space of 15 minutes. 1800X is more than sufficient to examine the reaction.

* In case you are wondering, skin is not supposed to "turn blue" when exposed to water.

additional skin samples (microscopic is sufficient) are now being sought out for comparison.

This will make the case that the entire skin of a human can be, and is likely affected by the presence of the CDB.
The case will, eventually, be extended to the entire body. Additional life forms (eg "The New Biology") will then come under review.

Okay this places an important and easily verifiable observation for you on the table.

Incidentally, the extent of influence and change with the dye CANNOT be seen by eye, ONLY under the microscope.

Ok, back to Conley. All of these circumstances along w/ the interaction stays that the alkyl halide assignment (tentative) must be seriously questioned. Iron + Cyanide derivatives are much closer to home these days. Let's see if we can find a spectrum of ferric ferrocyanide.

~~Turnan's~~ Turnbull's blue (~~Fe3+~~) (Fe2+)

Prussian blue Fe3+

One of the first things we see is that the wavenumber of absorbtion of ferricyanide / ferrocyanide changes as a result of concentration.

10M small peak @ 2115 cm^{-1} w/very strong @ 2040
1M weak peak @ ~2092
.1M " " "

~~However all con.~~
So concentration definitely affects peak magnitude.

The paper I am looking @ does not examine absorption in the 625 cm^{-1} region. This is my alternate but it still helps understand the 2040 region.

I did find one spectrum of sodium cyanide in Conley inorganic chapter and it does show absorption @ ~~2040~~ 2150 cm^{-1} and ~630 cm^{-1} so this puts us in range w/out doubt.

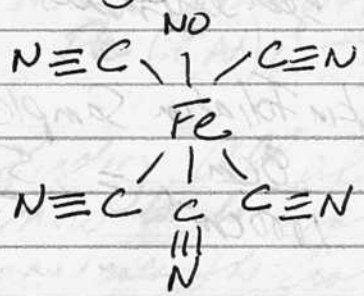
SDBS has potassium hexacyanoferrate
$C_6FeK_3N_6$
It has absorption peaks @ ~3450, 2120 and a very slight dip near 550 but not marked or noteworthy.

But there is Potassium, not iron.
$K_3Fe(CN)_6$

Ok, I did find something closer
Disodium Pentacyannitrosylferrate
It has absorption peaks
3630, 3548 2144 and @ ~ 640-650 cm^{-1}.

a definite strong peak in the region expected when Fe is attached.

SDBS reasonably close (a closest) match →



This says to me that we have extremely strong grounds to examine all "alkyl halide" cases near 650 +/- and reassign to a ferric ferricyanide compound. What are these cases?

✓ 3. ~~4~~. CDB Headspace S, Fe known
1. ~~2~~. CDB Pyrolysis S, Fe known, isothiocyanate known
✓ 4. Skin Flotation Sample Isothiocyanate known.
5. CDB Viscous Protein S, Fe known
6. Embedded Skin Crystal Isothiocyanate known

Next look @ the wavenumbers.

Let's pick up these peaks:

(3)(4) CDB Headspace: need to scale it.

$\frac{8cm}{1000cm^{-1}} = \frac{3.5cm}{x}$ $x = 437.5cm^{-1}$

$1043cm^{-1} - 437.5cm^{-1} = 605.5cm = \underline{606cm^{-1}}$

Best estimate.

(4) Skin Foliation Sample

$\frac{8cm}{1000cm^{-1}} = \frac{5.4cm}{x}$ $x = 675cm^{-1}$

$1283cm^{-1} - 675cm^{-1} = 608cm^{-1}$

(5) CDB Viscous Protein: $\leq 625cm^{-1}$

(6) Embedded Skin Crystal $\leq 649cm^{-1}$

(7) CDB Pyrolysis $669cm^{-1}$

We may add the

Environmental Filament $625cm^{-1}$
Aug 25 2017
Digested

$\frac{8cm}{1000cm^{-1}} = \frac{3cm}{x}$ $x = 375cm^{-1}$

$1000cm^{-1} - 375cm^{-1} = 625cm^{-1}$

We most certainly have consistency in our result w/ the variety of samples.

$\bar{X} = 631\ cm^{-1}$

$\sigma_s = 28.0\ cm^{-1}$

$n = 7$ different sample types.

I would say we have it. We are smack in the middle of the range of Conley's absorption for ferricyanide $Fe(CN)_6^{-4}$

This is a most profound finding. All alkyl halide assignments are now to be replaced. This also dramatically increases the role of iron & cyanide in the assessment.

We now have a revised ordered "Interest List"

		%
1. Sulfur / Related Compounds	12	100
2. Amides	6	50%
3. Iron	6	50%
4. Ferricyanide Complexes	6	50%
~~5. Thiocyanate Complexes~~	~~6~~	~~50%~~
~~Cyanide Derivatives~~		
5. & Aliphatic Hydrocarbons	6	50%
6. Thiocyanate Complexes, Cyanide Derivatives	5	42%
7. Esters (esp. sulfuric)	5	42%
8. ~~Amines & Aldehyde~~ Cyclic HC's	3	25%
9. Amines & Aldehydes	2	17%
10. Nitro & Ester Groups	1	8%

See Revision Ahead

Also see notes ahead – Revision #3.

An Important Summary:

* Hopefully you have some understanding of how serious the problem has become by now.

* Numerous sample types have been used to arrive @ the assessment of chemical signatures that are common:

* CDB, Env Filaments, Skin, Air, Water, CDB Probings

* Iron sulfur proteins & ~~ferrocyanide~~ & esters (esp S.) thiocyanates Complexes appear to be @ the root of the biochemical nature.

* Sulfur Esters are to be examined further for their properties & toxicity. Sulfate esters

* It is not unrealistic to posit that every square micrometer of skin of essentially the entire human race (and? Use The New Biology) is affected by this situation.
  Internals to the body are anticipated to be subject as well.

Revision #3

Continuing, and still thinking.
I will propose an alternative and simpler scenario now. If you look @ Conley's inorganic chart even a little further, there is one other feasible sector that has absorbance in this region.

IT IS THE SULFATES.

SO_3^{2-} ~(620-680) Very Strong $\bar{X}$=640

and

SO_4^{2-} ~(580-640) Medium $\bar{X}$=610

Now, guess where we are? Our $\bar{X}$ is 637 cm^{-1}
and our range was 606 to 675.
That is pretty darn close to SO_3^{2-}

Now here is another thought. You have already tested positive for sulfates in the CDB viscous protein so you know that you have this. In addition, you have only seen the blue pigment formation in well developed cultures or in biological samples; never in original CDB samples at the lab. They see the blue pigment as a more sophisticated development in the biological development.

In addition, I will always seek out the most benign & simplest explanation for the spectral result, and in this case it will be SO_3^-, especially since we already know that sulfur dominates essentially all spectra.

Additional Important Comments on 637 cm^{-1} Absorption:

* I shall now reveal the ordered list once again and shift all ferric ferricyanide assignments to SO_3^{2-}.

* The Annotation justification is the intensity of the absorption. The SO_3^{2-} is very strong absorption AND SO IS OURS.

* These factors make this to be a reasonable and conservative interpretation of the spectra that does not unnecessarily or unjustifiably introduce additional alarm or concern on the part of the public. If later revision is required it can always be made.

* Furthermore Caley does not state the strength of $Fe(CN_6)^{-4}$ absorption, the SDBS plot did not show very strong absorption (it might have been strong, but not very strong, I will check this) and the absorption range is skewed a bit to the right of the center closer to 625 cm^{-1} vs our 637 cm^{-1}.

* This seems to be a prudent & consistent decision to make.

* We are almost certain that we do have ferric ferricyanide in the picture and equation but all evidence indicate that it comes later w/ in the biological development cycle.

Revision: Interest List #3 06/30/18

The sulfide spectrum (Conley p 191) shows a very strong absorption peak indeed @ ~ 640 cm^{-1}. The sulfate absorption is weaker and @ ~ 610. Exactly as assessed. The sulfide interpretation is the more cautious route.

The changes are order once again

	n	no
1. Sulfur & related compounds	18	100
2. Amides	6	33
3. Iron	1	6
4. Aliphatic Hydrocarbons	6	33
5. Thiocyanate Complexes, Cyanide derivatives	5	28
6. Esters (esp. sulfate)	5	28
7. Cyclic hydrocarbons	3	17
8. Amines & aldehydes	2	11
9. Nitro & Ether groups	1	6

Our interest ordered group now becomes:

	Order of Health Concern
1. Sulfur & related compounds	4
2. Amides (Protein)	2
3. Aliphatic Hydrocarbons	6
4. Thiocyanate Complexes, Cyanogen	1
5. Esters (esp. sulfur based)	3
6. Cyclic Hydrocarbons	5

~~The system~~ Actually we can account for the methemoglobinemia question w/ the more developed form of growth that IS producing ferric ferricyanide (Prussian blue)

Melting Point Analyzer - Capillary tube instrument has been acquired - very helpful.

Sucrose (table sugar) will be used as example.

Capillary tube has been successfully closed on end and packed successfully.

We are running @ ~ 2°C / min.

Sweat
Start 149°C
Stop

Range of melting as an indicator of purity.
<2° most likely pure
5° + not pure, is a mixture
Melting point will always be a range.

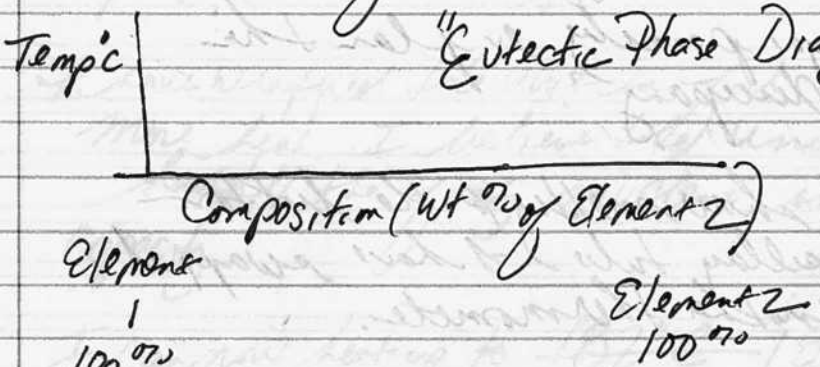
* Mixtures have a melting point that is LOWER and WIDER than the original compounds. This is quite fascinating.

The low heat setting will only heat to a max of ~ 100°C no matter how high you turn it up.

Now running @ high setting.
At this setting heater must be on.

Do not let the plastic light housing touch the heating unit.

Eutectic Point is an interesting topic.
Phase weight fraction - alloys, etc



The two phase condition (liquid + solid) is where the eutectic comes from.

The lowest temperature reached identifies the eutectic point of the mixture.

For tin, the eutectic point is 183°C of an example of Lead & Tin alloy.

The heater is really slowing down @ 148°C. (?)

We may need to place it closer to the wall.
At this rate a max temp of 150°C.

Very interesting result here.

Sucrose (table sugar) has a listed melting point of 186°C.

I showed initial melting @ 149°C.

There a radical difference.

How can ya possibly explain this large discrepancy?

I clearly show melting @ the bottom of the capillary tube & have swapped out my another thermometer.

First off, we learn that our oven thermometer was off by much more than it should be.

Truth is it is still in the process of melting @ 171°C.

So it actually is not melted yet even @ 173°C.

The oven thermometer require major Calibration.

They also indicate that the sugar may not be entirely pure pure.

It is actually only now that I am able to melt completely.

Thermometers are ranging from 169 to 173 $\bar{x} = 171°C$ vs actual of 186°C.

Either thermometers are incorrect (less likely w/ 2 of them) or the table sugar is not pure (more likely).

I have wrapped the unit in foil to contain more heat. I believe the unit can handle the fairly modest increased capacity.

I can now heat up to ~~182°C~~ 183°C, then is better.
The sugar has now all just barely melted @ 182.5°C. This is acceptable.
The newly melted sugar is clear, the sugar under extended heating has now turned amber in color.

One lesson is that we must calibrate the thermometers.

The oven thermometer (#1) reads 176°C so it is off considerably.

The digital multimeter thermometer reads 193°+

This is the only accurate thermometer I actually have.

I need accurate thermometers.
Even bulb thermometers can work if need be.

I can heat to a maximum of 195°C (~385°F) if need be.
This is not ideal but I did have hopes of heating to ~300°C.

You require a very good thermometer to extract any value from the process.

It looks like the Capillary tubes can be cut in half and are therefore less likely to break.

No they can not be cut in half but it is a good idea to reduce them by 1/3.

I am repeating the test w/ a fresh sample and a good thermometer.

@ 135°C it's already clearing translucency.
140 slightly more transparent.
150°C slightly transparent. Slowing the unit down.
155° E=O still slightly transparent
160° 6° per minute now. Slow further
Did a only halfway w/ foil now & much better
165 translucent.

169° first slight bubble @ bottom

171° bubbles @ bottom slightly layer.

186°C is the actual temp.
You slowed it down too early. Still translucent
@ 173°C, Bubble was a false alarm.
175°C Transparent.
176°C Translucent

182°C Crystals are starting to collapse
183°C Crystals collapsing
184°C Crystals collapsing further; first sign of L

185° Increased transparency now
some melting positively taking place
186° Almost all completely transparent now (melted). I am near max of instrument.
186.1 I say all melted.
This is perfect. 186.1 vs ~~186.5~~. 186 Theoretical
Superb result.

Jul 05 2018

I have worked out some good systems for both melting point & boiling point determinations.

Sucrose trial in place.

173°C Initial translucence appears
175° - 176.5 Δ = 1.5°/min
177° C Continued slight translucence
178° - 179.6 1.6°/min
181°C Continued slight translucence -
183°C same
184°C same
185°C same
185°C decomposition starts @ 185.1
185.2 start
185.5 decomposing
185.6 "
185.7 first liquid
185.0 Definite liquid - first melt
186.0 ½ melt
186.5 75% melt
186.7 85% melt
187°C full melt

Ok, once again, that was a perfect result

Melting point of sucrose is 186°C.

I have 2 separate methods of melt point determination.

I now have a capillary device available. It works great except that it is designed for 220V instead of 120 therefore I am only getting a max temp of 160°C which is not sufficient.

It is rated to 300°C but since it is from India, I understand this now means 220V.

The problem has, nevertheless been solved. The addition of a propane torch @ a very low setting allowed to heat gradually the heat block area is working superbly to supplement the heat input as required. The heat flow combination from both sources rheostat & torch can be configured to control the melt quite precisely. The should be able to get up to 300°C as needed.

Without the torch supplement, we are good to 160°C which is adequate for low melting temperature. An excellent thermometer is paramount to good results.

The alternative method use a heating block. This is extremely simple and also sufficient.

The ideal setup does include a sand bath along w/ the torch and excellent thermometer but it can also work fine. The main loss is that of lighted magnification of a very small sample and the ability to use capillary tubes for a highly controlled result.

The electrical heater w/ the torch supplement is preferred but it does have a larger foot print. Heat protection of the table will be increasingly important.
The system requires

1. Capillary tubes
2. Torch & Canister
3. High quality thermometers (Boiling Point Taylor Mod, Thomas (2), High Temp (2))
4. Magnifying glass
5. Vice
6. Light (Top light)
7. Baking pan to contain torch
8. Styrofoam brace for torch
9. Tongue to assist rate of heating.
10. Brass Dropping Tube.
11. Boiling Wire for BP work.
12. Syringe & Solvents
13. Timer

The heating block method requires

1. The sand bath in the baby pan (preferred)
2. Heating block
3. High quality thermometer
4. Magnifying glass
5. Timer
6. Torch & Cannette
7. Styrofoam brace for torch
8. Adequate light also.

They are not sufficient. Electrical heating w/ lighted magnification is preferred. Electrical heat can use torch alone if required.

Another advantage of the electrical heat / torch combination is that it can also accurately be used as a boiling point instrument. This is still under development. The greatest difficulty he appears to result from the thickness of the temperature probe, only a very fine probe will be small enough to allow a reading of temperature. This is a significant restriction.

The method also require the development of narrow custom formed boiling test tubes, also because of the restriction on the heating unit. This will be further tested.

Boiling Point Assessment.

We start w/ acetone.
6 cm tube, 1/2 full.
Starting temp 36°C.

Good news is that I can actually stick the temp probe into a Capillary tube and it should hold.

But guess what, that is not the problem. It must fit into the boiling tube. Only the digital multimeta probe will fit the thermo.

Question: does the probe need to touch the liquid or can it measure the vapor? We will see.

33.6°C start.
You can actually see the liquid in the lighted magnifier.
41°C
44°C
52°C Block Temp 59°C

At the evaporating add solution.
51.7°C → 62°C Block

Probe just above liquid level.

Probe is now touching liquid.
When bubble pops, it settles on 58°C

58°C bubble 70°C block

Evaporate quickly. Inserted "boiling wire" instead of
slowly clip. Bubble gave 51.5°C all evaporated

~~51.7~~ 51.4° } The heating vapor is giving
51.4° } you the accurate temperature
51.4

BP of acetone @ sea level is 56.2°
Δ sea level = 56.2
- 51.4
4.8°C

Stable readings each time. I am not sure
the "boiling wire" helps anything. Now for water,
it is helpful to have 2 separate thermometers
for the block temperature.

One good thing about the method is that it uses
very little solution. Acetone is especially volatile
so it is expected to be more difficult.

85°C bubbles are seen.

Ok, the heating wire is absolutely helping
stabilize.

Jul 06 2018

You are getting extremely stable results w/ the Crosley ware. The method is to have the Crosley liquid statulized by the Crosley ware heater the temperature probe intermittently w/ the heated fluid. If the probe responds instantaneously as this one does, you get exceptional results.

Boiling temp $\cong$ 94.5°
Δ steal level = 100° − 94.5° = 5.5°
Acetone Δ = 4.8°
So Δ = 5° appear to be close in both cases.

You actually only need a small amt of liquid to produce the vapor.

Now for xylene. Mold test support added. The temperature probe needs to be immediately above the liquid. The test will increase the fire hazard when volatiles are examined. Splash guard has been added.

128°C bubbles activity starts.
127°C
125°C
131°C
131.5
131.2
131.5

When you adjust the tube you are getting different readings! You must find a position of the probe that creates a maximum temp reading.

132.1
132.5
132.5
132.5

These are very stable results @ the maximum stable temp reached w/ xylene.

If you estimate a ±5° sea level differential then leads to an estimated BP temp for xylene of 132.5 + 5 = 137.5° C.

With the "boiling wire" there was no immediate fire hazard or uncontrolled boiling.

This is our result. The method seems to work quite well. The 6 cm tubes work well.

138.4° C is listed for p xylene.

This is excellent work.

1. Acetone
2. Water
3. Xylene

} have all succeeded very well w/ the method.

Your remaining problem is the variety of temperature probes.

Alternative thermometer use - taped on.
What about small hose clamp?
Block temp 111° Tube temp 49°

What if we punch a hole and hold the probe @ the top of the tube w/ the vice.
Block: 122°C / Tube Temp 62°C

The probe did not get hot enough. It only made it to ~ 100°C. It appears the probe must be immersed in the vapor.

—

I have done something quite ingenious. With the Dremel tool, I have removed a sufficient amount of the probe exterior housing, & now see the internal probe filament exposed. I'll now slide it easily into the boiling point tube. This is great. Now we must be concerned about the stated range of the thermometer which is stated to be a max of 150°C.

However, upon cheek removal of the housing, I saw a temperature rise to 200°C. Thermometer calibration may also be required.

I have reached a maximum stable temp of 131.5°C. (vs 132.5°C).

I love it.

I have created an inexpensive version of the probe thermometer that is easily portable and can also be calibrated if need be, however it appears to be sufficiently accurate. This is marvelous.

The probe will fit and work in all situations, both melting and boiling trials. Great work Clyford.

This was ingenious. Save the housing for the thermometer & no longer need the large & bulky digital multimeter for modest temperature measurement (only high temp work).

Copper sulfate melts @ 110°C - an easy target.

DMSO boils @ 189°C - another good target and I have some.

You need to be gentle w/ the suction applied on the syringes.

DMSO boiling is in progress.
176°C under observation.
184°C " " 223°C It is boiling. I am too high.
193°C " "
200°C " "
206°C " "
218°C " "

OK, the sample produced some Complication. First off, a high temp boiling compound needs more material in the tube. It does not explode in any way and you need to see the liquid by eye.

It is fine to have the probe immersed in the liquid, either immediately above the liquid level or in the liquid is sufficient.

The thermometer appear to have held up to ~270°C - There is somewhat remarkable & unexpected since supposedly it was only rated to 150°C.

A definite boiling point was reached (you have to see the liquid for a high temp cmpd).

Small bubbles on the boiling wire are formed @ ~215°C. The boiling wire is different, however, than the boiling point. You must wait until you have a stable max temperature.

Now @ 240°C.

We have it. We have a maximum stable temperature of ~340°C.

You must have had this set for F°
~171°C need 189°C

I never expected the thermometer to handle the heat it has.

The temperature of the liquid is quite astounding. This is the highest level ever expected to be used in this device.

I do not think the DMSO identity is quite so confirmed now.

I am interested in repeating the trial. Is heating changing the nature of the compound?

What I have certainly done here is create an effective and material efficient microscale method of boiling point determination.

The method also combines equally well with that of melting point determination.

Our DMSO problem is a classic case of the need that has risen to measure
1. Melting point
2. Boiling point
3. Index of refraction.

I am repeating the trial. I may have had wrong scale on thermometer.

81°C We see some bubble formation, this is due to the "cooling wire" - it works well. This is a very viscous fluid under examination.

The new thermometers have arrived. They look to be an excellent value.

Be careful to not set the face of the Taylor thermometer too hot or it will turn black & lock up / be unreadable.

DMSO:
Block 171°C Tube: 142°C
Break bubbles from cooling wire.

Block 181°C Tube: 153°C
The thermometer face is getting too hot and dark & unreadable. You must enter it manually upon suitable cooling.
Block: 195°C Tube: 154°C

The more viscous the sample liquid, the more volume to be added to the cooling tube.

Block 223°C Tube 155°C

Notice that we have a stable temperature being reduced. A lower temperature indicates a lack of purity – quite possible in this case.

216°C Block	171°C Tube
223°C "	171°C "
220°C "	173°C "

Turn the thermometer face away from the heat source.

230°C	178°C
231°C	181°C
233°C	183°
235°C	184°C
238°C	185°C
238	186°C
237°C	187.5°C
Theoretical	187°C
189°C Good Job. It is DMSO.	

Ok, we caused some damage to the unit. We melted the dial. It should be metal instead of plastic.

Ok, we are melting our pad also.

The good news is that indeed we did find the

The magnifying glass popped out also.

Prepared the damage.
I have made a brass-wood temp lever that will not melt now.

I have there no need for the magnifying glass that was built in. The use of a full size magnifier (with a vice holder) gives a better and clearer image regardless

The unit has now been battle tested to ~ 280°C. There is quite decent. The new thermometer works extremely well and is considerably more portable than the multimeter.

Testing sucrose again w/ the Capillary tube.

Sequence on the Capillary tube is
P. Seal one end
1. Fill a (a load) a full size tube
2. Pack it @ the bottom
3. Break the tube in half
4. Seal the broken end by the remainder piece
5. Pack & reweigh.

Sucrose theoretical is 186°C

Sucrose Test: - Thomas Thermometer

166°C	Solid
180°C	Solid
182°C	"
184°C	"
185°	Starts to melt.
186°	50% melted
187°	50% melted
188°	almost appears solidified
189°	glossy (it is all liquid)
190°	all liquid

Oh, just melt was indeed the point.
~186°C right on track.

The mean the thermometer is spot on.

Melting Point. DMSO tube all melted @ 26.7°C

CuSO4 melting point determination - Capillary.

115°	Observed Solid
121°	Beginning more translucent.
137°	More translucent
145°	Discolored, but still appears solid
168°	Solid

Jul 07 2018

Continuing w/ $CuSO_4$. Results not what is expected from jar.

Viewed sample under binocular dissecting microscope. It never did melt.

170°C	solid	above 250° it becomes a cave for the solid melting block
201°C	"	
234°C	solid.	

250°C solid. NO MELTING

shift to melting block w/ high temp thermometer.

225° discoloration & signs of burning around the edge.

250° solid as above
360° solid.
350°C solid.
400°C solid.
515°C solid
550°C solid
600°C solid.
620°C solid

$CuSO_4 \cdot 5H_2O$ does not melt.

What does happen is:

1. @ ~ 225°C it loses its color, appears to have been oxidized.

2. It never melts to ~ 620°C

3. Direct torching appears to further completely oxidize the material, leaving an ash-like result.

There is a stark contradiction to the stated melting point of 110°C. Remember that we have $CuSO_4 \cdot 5H_2O$, not $CuSO_4$.

All in all, quite the surprise, and it shows the value of direct experimentation.

Jul 08 2010

I have now improved the combined melting point – boiling point apparatus for improved flexibility & safety.

I have improved the lighting and magnification of the sample. & have decreased the vulnerability to heat damage to the unit.

I will set a limit of 250°C on the use of the device, for both melting & boiling.

The thermometer retainer has also been improved.

Going above 250°C requires the melting block (no difficulty w/ that) but it also requires the large multimeter temperature probe. That is not convenient.

I have 2 high temp thermometers on schedule for arrival.

We are evaluating performance once again w/ sucrose. Calibration run.
I can see the sample properly now.

Theoretical = 186°C

°C
161.5 solid ~2°/min
175°C solid 3°/min
182°C "
183° slight translucence @ bottom
184°C "
185°C "
186°C 1st melting trace starts
186.5 " .
187°C 50% melted
188°C 65% melt
188.5 100% melt.

We know from experience that start of melt to 50% melt is sufficient to encompass the range.
The plateau @ 186.5 - 187°C

vs Theoretical 186°C - Excellent work and method is now in place.

An interesting question coming up is whether we can determine melting or boiling point solution concentration levels of our micro boiling tube. We see that we can take medicine 0.37 grams easily but we actually do not know the volume until.

3.29 mm
42.19 m

= 0.37 gms?

Let's see if we can determine volume of the boiling tube w/ a micrometer.

Diam = 3.29 mm
Volume height = 42.19 mm

$V = \pi \cdot r^2 \cdot L = 3.14159 (\frac{3.29\text{ mm}}{2})^2 (42.19\text{ mm})$

$= 358.66\ m^3$

$= .359\ cm^3 = 0.0.359\ cm^3$

$= 0.36$ gms H_2O vs measured 0.37 gms.

This is quite good. 2.7% error. Reasonable and expected, actually better than expected.

Now the question is, can we determine the MW of sucrose by the boiling point method?

46.32 gms H_2O
3.92 gms sucrose added
$\Sigma =$ 50.24 gms total

Molar Concentration:

$\frac{3.92\text{ gms}}{46.32\text{ gms } H_2O} = \frac{X}{1000\text{ gms } H_2O}$

X = 84.629 gms

84.629 gms
342.3 gms/mole (Molecular wgt of sucrose)

= 0.247 Molar solution of sucrose.

Now, the question is, how much does the charge the boiling pt of water?

First determine the BP of H_2O @ Monticello UT @ ~7000'.
A good thing is that BP does not depend upon volume.

Do not forget the boiling wire.
Prepare a spare boiling thermometer.

The temperature result is not as stable as I would like it to be.

~~The vapor pressure stabilizes @ X94°C. Block temperature ~ 120°C.~~ That is, X94.0°C.

We are having problems with the thermometer and the solution is not clear or pure
Vapor pressure maximized @ ~~95.6°C~~
97.1°C
97.4°C

Upon heating, it stabilized @ 95°C.
We obviously have a problem.
The results are not stable enough.

The boiling temperature was not stable enough for boiling point determination.

Go are increasing the scale of the problem. You are using a larger test tube and beaker now.

Ok, we are all over the map right now. Obviously we have some problems. Water in a test tube (w/ a boiling chip) has boiled @ 87.1°C. What exactly is taking place here. Repeat.

I have replaced the boiling chip w/ a piece of wire to avoid mineral contamination.

The result now w/ the best thermometer is 94.2°C. The does appear reasonable.

Repeat. 94.8°C A cleaner test tube was w/ greater volume.

Boiling wire was almost lost.

Repeat trial: 94.8°C

We now have stable results.
The melting point apparatus can be used, but it is indeed more sensitive to heating variations. The most accurate reading appears to occur w/ very little liquid in the tube (i.e. a few drops) and attempt to measure the highest vapor pressure achieved. It does not, however, appear to be accurate enough, certainly for molecular wt. determination.
(We do get ~94.1°C but it is more variable (94.5° — 94.4° – 94.9°C)

Now for the sugar water:
94.1°C
K_b = molal boiling point constant = 0.512°C/mole

Molar mass = $\frac{\text{Solute (gms)}}{\text{x Moles}}$ Molality = $\frac{\text{Moles}}{\text{kg of solvent}}$

We know that we have 0.241 molar solution of sucrose
i.e. $0.241\left(\frac{342.3\text{gms}}{\text{liter}}\right) = \frac{84.54\text{8 gms}}{\text{l}} = \frac{84.548}{1000\text{gms } H_2O}$

$\Rightarrow$ molality $\approx$ .0845 molality.

$\Delta T = mK_b$ $\quad m$ = molality.

$m = \frac{\Delta T}{K_b} = \frac{0.7^\circ C}{.512} = 1.367$ molality

$= \frac{1.367 \text{ moles}}{\text{kg of solvent}}$.

$\Delta T = m K_b$ (boiling)

$\Delta T = m K_f$ (freezing)

$\text{Moles} = \frac{1.367 \text{ moles}}{\text{kg of solvent}} \cdot .04632 \text{ kg of solvent}$

$= .0633$ moles

$\text{Molar mass} = \frac{3.92 \text{ gms sucrose}}{.0633 \text{ moles}}$

$= 61.93$ gms/mol

which is way off. It should be 342.3 gms/mol
Error factor = 5.5. ??

Ok, the problem is that the addition of a non-volatile solute to a solvent Causes the boiling point to increase! Not decrease.

So why did it decrease for us..??
It should have risen by the rule of ~~+0.14 degrees~~. $+0.127^\circ C$

So why did this not happen??

First of, the method is simply not accurate enough to determine the molar mass. But the boiling pt of the sugar water should have nevertheless been higher than pure water alone.

We have a very strange result here. BP of sucrose water is 94.0°C - repeated vs 94.6°C water - repeated. It should be higher???

Also, you can indeed determine the BP of water w/a the melt point apparatus if you water the process carefully. The cooling wire is indeed important. The best result is when the probe and wire are subject to heat loss from the cooling process.

You should easily be able to determine BP w/in a degree or so.

BP raising, however, is not an accurate method to determine molecular mass, and we somewhat knew that already.

Here the desire to get the osmometer working here, hopefully by this fall.

- Guess what, the sucrose solution has now increased to 94.7°C, so it is getting closer, but still too low.

Now guess what: We boil the sucrose solution up a third and we immediately pull it up to 95.1°C OK

So we need to force the result to bring up to full heat.

With very careful use of the torch we get 94.9°C We are in business now

$\Delta T = m K_b \quad m = \frac{\Delta T}{K_b} = \frac{0.2^\circ C}{.512} = 0.39$ molality

and moles $= 0.39 \frac{\text{moles}}{\text{kg } H_2O}$ (.04632 kg H_2O) kg

$= .0181$ moles

and molar mass $= \frac{\text{solute}}{\text{moles}} = \frac{3.92 \text{ gms}}{.0181 \text{ mols}}$

$= 216.6 \frac{\text{gms}}{\text{mol}}$

vs theoretical ~~344~~ 342.3 gms/mol

We are now in range. Thermometer must be able to measure to .01°C

$m = \frac{\Delta T}{K_b}$ $\quad \Delta m = \frac{1}{K_b}(\Delta(\Delta T))$

$\Delta m = \frac{1}{.512}(.05^{\circ}C \text{ error}) = .098 \cong .1$ error in molality

This means our molality could easily range from .3 to .5

Assuming .3 : $.3(.04632) \Rightarrow .014 \Rightarrow$

$\frac{3.92}{.014} = 280 \frac{gms}{mol}$ or

Assuming .5 $\quad .5(.04632) = .023$

$\frac{3.92}{.023} = 170 gms/mol$

So our answer is ~ 215 gms/mol vs theoretical 340

with a low expectation of 170gms (Error $\cong$ 50%)
and a high of 280gms (Error = 18%)

This is now all reasonable.

Oct 31 2018

Back in Monticello, UT @ the lab.
Anticipate to be here ~ 2 mos.

Approx 4 boxes of samples have been received from citizens.

The feed involves the airborne (helicopter) deposition of materials onto an organic farm.

Apparently the preliminary report via EPA (informal) is that the material is highly water absorbing and used for erosion control.

Even if this turns out to be the case, hardly acceptable on private lands without consent or notification, and especially w.r.t. to an organic farm.

Material is greenish, and of a paper consistency in a pellet form.

I have offered to study the nature of the material.

I will start w/ solubility tests.

" "

Polyacrylamide is the purported nature of the material.

UNITED STATES DEPARTMENT OF AGRICULTURE
NATURAL RESOURCES CONSERVATION SERVICE

CONSERVATION PRACTICE STANDARD

ANIONIC POLYACRYLAMIDE (PAM) EROSION CONTROL

(Acre)

CODE 450

DEFINITION

Erosion control through application of water-soluble anionic polyacrylamide (PAM).

PURPOSE

This practice is applied as part of a conservation management system to support one or more of the following:

- Minimize or control irrigation-induced soil erosion.
- Reduce wind and/or precipitation erosion.

CONDITIONS WHERE PRACTICE APPLIES

- On irrigated lands susceptible to irrigation-induced erosion, excluding peat soils, and where the sodium adsorption ratio (SAR) of irrigation water is less than 15;
- On areas where the timely establishment of vegetation may not be feasible or where vegetative cover is absent or inadequate;
- On areas where plant residues are inadequate to protect the soil surface from wind erosion; and
- On sites where disturbance activities prevent establishment or maintenance of a cover crop;

This standard does not apply to the application of polyacrylamides to flowing, non-irrigation, waters.

CRITERIA

General Criteria Applicable to All Purposes

Changes in management shall be implemented where increases in soil infiltration rates are a result of implementing this practice.

The polyacrylamide (PAM) shall:

- be of the anionic type meeting acrylamide monomer limits of ≤0.05 percent (%),
- have a charge density of 10 to 55%, by weight,
- have a molecular weight of 6 to 24 Mg/mole,
- be mixed and/or applied in accordance with all Occupational Safety and Health Administration (OSHA) Material Safety Data Sheet requirements and the manufacturer's recommendations for the specified use, and
- conform to all federal, state, and local laws, rules, and regulations.

Additional Criteria Applicable To Irrigation Induced Soil Erosion

Surface Irrigation

PAM shall be used during the first irrigation and after any soil disturbance (pre-irrigation is considered irrigation) and during later irrigation if soil movement is observed.

Mixed concentrations of PAM shall be added to irrigation water only during the advance phase of a surface irrigation. The advance phase shall be considered the time irrigation starts until water has advanced to the end of the field.

NRCS, Alabama
October 2001

Dry or "patch" treatments of PAM shall be placed over an area of the first five (5) feet of furrow.

The resulting concentration of PAM in irrigation water shall not exceed 10 ppm of pure form polyacrylamide, applied on a total product basis.

Sprinkler Irrigation

The maximum application rate of polyacrylamide active ingredient shall not exceed four (4) pounds per acre (lb/ac) per single application event.

PAM mixtures will be totally mixed and liquefied prior to injection into the irrigation system.

Injection shall occur on the downstream side of all screens and/or filters and conform to all federal and state chemigation standards.

Additional Criteria Applicable To Reduce Wind and/or Precipitation Erosion

The maximum application rate of pure form polyacrylamide shall not exceed 200 lb/ac per year.

Emulsion batches shall be mixed with pure form polyacrylamide not exceeding 200 pounds per batch.

Application method shall insure uniform coverage to the target area, minimizing drift to non-target areas.

CONSIDERATIONS

The following relate to the application of the polyacrylamide practice that may enhance, or avoid problems with the practice but are not required to insure its basic conservation function.

General

PAM application rates may need to be adjusted based on soil properties, slope, and type of erosion targeted.

Where reasonably possible, tailwater or runoff containing PAM should be stored for re-use or recycled on other land areas.

Use of polyacrylamide in combination with other conservation and Best Management Practices will improve erosion control.

Irrigation Induced Erosion Considerations

Other conservation treatments such as land leveling, irrigation water management, reduced tillage, reservoir tillage, crop rotations, etc. should be used in conjunction with this practice to control irrigation-induced erosion.

PAM may result in an increase in surface irrigation infiltration of up to 60%, with 15% being typical on medium textured soils.

To compensate for PAM changes in infiltration, adjustments in flow rates, time of set, and tillage practices should be considered.

Adjustment from maximum PAM rates and volumes should be considered so long as no visible erosion occurs.

Secondary applications on undisturbed soil may be needed in surface irrigation when sediment or erosion is noted.

Sprinkler systems will likely need multiple applications to achieve a significant erosion reduction.

For sprinkler systems, before and after injecting concentrated liquid PAM (30 to 50% active ingredient) into sprinkler irrigation systems, it is a good practice to pump a surfactant (crop oil) through the injection system (pump, tubing, valves, etc.). Surfactants provide a buffer between PAM and water so non-flowing PAM does not contact water and form a gelatinous mass that can plug valves and tubing.

For sprinkler injection, the injection pump should be started after water is flowing in the sprinkler system and stopped when the irrigation pump stops.

Applications at the end of the season are discouraged, unless the field has been recently tilled.

Wind or Precipitation Erosion Considerations

Adding seed to polyacrylamide mixture may provide additional erosion protection beyond the life of the PAM material.

PAM may improve water quality, infiltration, soil fertility, and air quality.

Safety and Health

Use proper personal protective equipment, e.g. gloves, masks, and other health and safety precautions in accordance with the label, industry, and other federal or state rules and guidelines.

If inhaled in large quantities, PAM dust can cause choking and difficulty in breathing. Persons handling and mixing PAM shall use a dust mask of a type recommended by the manufacturer.

PAM solutions can cause surfaces, tools, etc. to become very slippery when wet.

Clean liquid PAM spills with dry absorbent material (sawdust, soil, cat litter, etc.) and sweep/collect dry PAM material without washing with water.

PLANS AND SPECIFICATIONS

Specifications will be developed site specifically for each application. Specifications for this practice will be prepared for each field or treatment unit according to the criteria, considerations, and operation and maintenance described in this standard. Specifications shall be recorded using approved specification sheets, job sheets, narrative statements in the conservation plan, or other acceptable documentation.

OPERATION AND MAINTENANCE

An operation and maintenance plan must be prepared for use by the landowner or operator responsible for PAM application. The plan should provide specific instructions for PAM applications to insure it is used properly. Plan items may consist of:

- Reapply PAM to disturbed or tilled areas, including high traffic use areas.
- Monitoring advance phases of the irrigation to assure applications are discontinued when runoff begins.
- Equipment is operated and maintained to provide uniform application rates.
- Maintenance of screens and filtering facilities.
- Rinse all PAM mixing and application equipment thoroughly with water to avoid formation of PAM residues.
- PAM is a flocculating agent that may cause deposition in downstream watercourses or other locations when it comes in contact with sediment-laden waters. Downstream deposition from the use of PAM may require periodic cleaning to maintain normal functions.

REFERENCES

Looks like source of identification req. is USDA

One thing is already clear; the material in no way appears to be water soluble.

Let's do some research on appearance.

They are apparently called "Earth Guard"
Product name.

Haley Brown is the journalist on the issue.

The photograph on the adjacent page is accurate and faithful. The material is greenest to greenish blue.

Tera Nova is the company of manufacture.

The fibrous material is a matrix material, apparently of cellulose / wood fiber.

This is why it is not dissolving in water. However, I anticipate water soluble compounds are released.

"Hydro mulch" appears to be the trade name for the matrix material.

Next, the MSDS has been located.

MVP pellets sent for testing

4:31 pm TOP STORIES

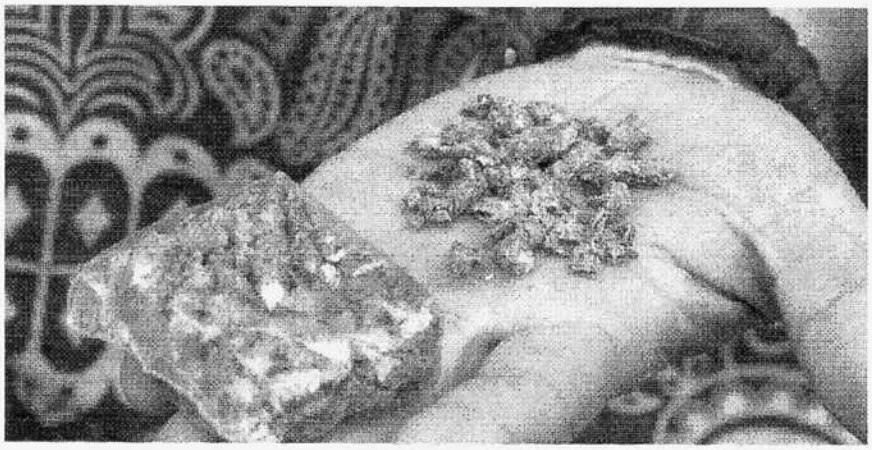
(Summers County) The owners of Blackberry Botanicals, an organic herb farm, say they have sent the pellets dropped on their farm by Mountain Valley Pipeline to four different testing labs to evaluate the composition. Beth Laferriere says the results will be available at the end of the month.

The pellets, meant to prevent soil erosion, are called Earth Guard.

The concern is that the pellets may contain non-organic matter that has contaminated the organic farm and possibly the New River. The West Virginia Department of Environment Protection is also investigating the spill.

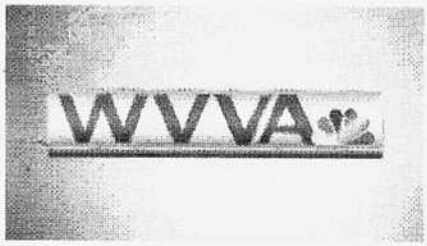
WVVA Weather Authority

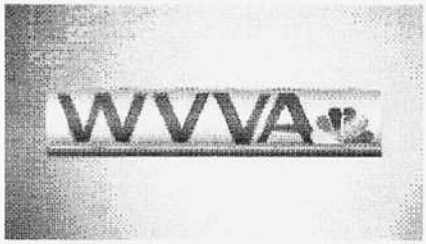
Wednesday Evening Forecast



Haley Brown

More News





(WVVA) Wednesday evening will be dry and mild for Halloween activities with temperatures in the upper 60s, falling into the

CONNECT WITH WVVA

TOP STORIES

Kids and parents Trick or Treat a day early in Hinton

October 2018: Kidnapping, murder among McDowell County indictments

Twenty-eight men and women have been indicted by the latest grand jury in McDowell County.

1 killed in McDowell County shooting

The West Virginia State Police is investigating a fatal shooting in McDowell County.

There are 3 main constituents to the application

1. Water
2. "Earthguard"
   "Anionic water soluble polymer in emulsion".

Note that the pp product does not biodegrade.

3. Hydro mulch, or the fibrous matrix

The combined materials are then sprayed.

A company video gives a general overview of the product and its application, from a marketing & sales perspective.

This was, and is, indeed my primary concern

Organic polymeric flocculants are widely used nowadays due to its remarkable ability to flocculate efficiently with low dosage. However, its application is associated with lack of biodegradability and dispersion of monomers residue in water that may represent a health hazard.

Polyacrylamides can eventually break down to acrylamides. There are potential health effects here.

WaterSolve, LLC

Dry Anionic Polymers
Solve 5240 Series

Material Safety Data Sheet

Date Issued: 04/13/2013
Date Revised: 04/13/2013

1. CHEMICAL PRODUCT AND COMPANY IDENTIFICATION

PRODUCT NAME: **SOLVE 5240 Series**
CHEMICAL TYPE: Cationic polymer in solution
COMPANY: WaterSolve, LLC. 5031 68TH Street Caledonia, Michigan 49316, USA
For Product information call 616-575-8693.
For Chemical Emergency Spill, Leak, Fire, Exposure, or Accident
Call CHEMTREC Day or Night
Within USA and Canada: 1-800-424-9300
Outside USA and Canada: +1 703-527-3887 (collect calls accepted)

2. COMPOSITION/INFORMATION ON INGREDIENTS

Identification of the preparation: Anionic water-soluble polymer

3. HAZARDS IDENTIFICATION

Aqueous solutions or powers that become wet render extremely slippery surfaces.

4. FIRST AID MEASURES

Inhalation: Move to fresh air.

Skin Contact: Wash off immediately with soap and plenty of water. In case of persistent skin irritation, consult a physician.

Eye Contact: Rinse thoroughly with plenty of water, also under the eyelids. In case of persistent eye irritation, consult a physician.

Ingestion: The product is not considered toxic based on studies on laboratory animals.

5. FIRE FIGHTING MEASURES

Suitable extinguishing media: Water, water spray, foam, carbon dioxide (CO_2), dry powder.

Special fire-fighting precautions: Aqueous solutions or powders that become wet produce extremely slippery surfaces.

Protective equipment for firefighters:
No Special protective equipment required.

6. ACCIDENTAL RELEASE MEASURES

Personal precautions: No special precautions required.

Environmental precautions: Do not contaminate water.

Methods for cleaning up: Do not flush with water. Clean up promptly by sweeping or vacuum. Keep in suitable and closed containers for disposal. After cleaning, flush away traces with water.

7. HANDLING AND STORAGE

Handling: Avoid contact with skin and eyes. Avoid dust formation. Do not breathe dust. Wash hand before breaks and at the end of the workday. When preparing the working solution ensure there is adequate ventilation. When using do not smoke.

Storage: Keep in a dry, cool place (0-30 deg. C). Keep away from heat and sources of ignition. Freezing will affect the physical condition and may damage the material.

8. EXPOSURE CONTROLS/PERSONAL PROTECTION

Engineering controls: Use local exhaust if misting occurs. Natural ventilation is adequate in absence of mists.

Personal protection equipment

Respiratory protection: In case of insufficient ventilation wear suitable respiratory equipment. Dust safety masks are recommended where concentration of total dust is more that 10 mg/m³.

Hand Protection: Rubber gloves.

Eye protection: Safety glasses with side-shields. Do not wear contact lenses.

Skin protection: Chemical resistant apron or protective suit if splashing or contact with solution is likely.

Hygiene measures: Wash hands before breaks and at the end of workday. Handle in accordance with good industrial hygiene and safety practice.

9. PHYSICAL AND CHEMICAL PROPERTIES

Form:	granular solid
Color:	white
Odor:	none
pH:	4-9 @ 5 g/l;
Melting point:	Not applicable
Flash point (deg.C):	Not applicable
Autoignition temp. (deg.C):	Not applicable
Vapour pressure (mm Hg)	Not applicable

10. STABILITY AND REACTIVITY

Stability: Product is stable. No hazardous polymerization will occur.

Hazardous decomposition Products:

Thermal decomposition may produce: nitrogen oxides (NOx), carbon oxides. (COx).

11. TOXICOLOGICAL INFORMATION

Accute toxicity

Oral: LD50/oral/rat > 2,000 mg/kg
Dermal: The results of testing on rabbits showed this material to be non-toxic even at high dose levels.
Inhalation: The product is not expected to be toxic by inhalation.

Irritation

Skin: The results of testing on rabbits showed this material to be non-irritating to the skin.
Eyes: Testing conducted according to the Draize technique showed the material produces no corneal or iridial effects and only slight transitory conjuctival effects similar to those which all granular materials have on conjunctivae.
Sensitization: The results of testing on guinea pigs showed this material to be non-sensitizing.
Chronic toxicity:
Two year feeding studies on rats did not reveal any adverse health effects. A two-year Feeding study on dogs did not reveal adverse health effects.

12. ECOLOGICAL INFORMATION

Fish: LC50/*Pimephales promelas* (Fathead minnows)/96h > 100mg/L (OECD203)

Algae: IC50/*Selanastrum capricornutum*/ 72h > 100 mg/L (OECD 201)

Daphnia: LC50/*Chaetogammarus marinus*/ 48 h = 100 mg/L (OECD 202)

Bioaccumulation:
Does not bioaccumulate.
Persistence/degradability:
Not readily biodegradable.

13. DISPOSAL CONSIDERATIONS

Waste from residues/unused products:
In accordance with federal, state and local regulations
Contaminated packaging:
Rinse empty containers with water and use the rinse water to prepare the working solution. Can be landfilled or incinerated, when in compliance with local regulations.

14. TRANSPORT INFORMATION

Not regulated by DOT.
Material not restricted for transportation by DOT, IMO, IATA regulations.

15. REGULATORY INFORMATION

RCRA status: Not a hazardous waste.
Hazardous waste number: Not applicable
Reportable quantity (40 CFR 302):
Not applicable

Threshold planning quantity (40 CFR 335):
Not applicable

California Proposition 65 information:
The following statement is made in order to comply with the California Safe Drinking Water and Toxic Enforcement Act of 1986: This product contains a chemical(s) known to the State of California to cause cancer: residual acrylamide.

All components of this product are on the TSCA and DSL inventories WHMIS (CANADA): Not Regulated

HMIS & NFPA Ratings	HMIS	NFPA
Health:	1	1
Flammability:	1	1
Reactivity	0	0

Reasonable care has been taken in the preparation of this information, but the manufacturer makes no warranty of merchantability or any other warranty, expressed or implied, with respect to this information. The manufacturer makes no representations and assumes no liability for any direct, incidental or consequential damages resulting from its use. Recipients are advised to confirm in advance of need that the information is current, applicable, and suitable to their circumstances. This information is for the specific material described only and may not be valid if the material is used in combination with any other materials or in any process. The user is responsible to determine the completeness of the information and suitability for the user's own particular use. The knowledge and belief of the company, the information is accurate and reliable as of the date indicated but the company makes no express or implied warranty of merchantability for the material or the information. The company makes no express or implied warranty of fitness for a purpose for the material or for the information. Users of any chemical should educate themselves on all aspects of its use by independent investigation of current scientific and medical knowledge that the material can be used safely

The issue of concern is the potential of the polymer to break down into the monomer form.

ie, note the MSDS reference to a

"residual acrylamide"

known to cause cancer.

Anionic means a negatively charged ion.

The wikipedia article does give a reasonable summary on the issue, including the environmental concerns and chemical structure.

All in all, as mentioned earlier, the material does not exactly satisfy the definition of benign, especially with respect to the high standards inherent within an organic farm.

The degradation of polyacrylamides is a significant further topic of research (eg, under alkaline conditions).

Terra Novo, Inc.
EarthGuard

MATERIAL SAFETY DATA

Revision Date: 10/3/2012

1. IDENTIFICATION OF THE SUBSTANCE/PREPARATION AND THE COMPANY

PRODUCT NAME:	**EarthGuard**
COMPANY:	Terra Novo, Inc., 2930 Patton Way BAKERSFIELD, CA 93308, USA
TELEPHONE:	661.587.5716
EMERGENCY PHONE:	CHEMTREC 800.424.9300
PRODUCT USE:	Processing aid for industrial application

2. HAZARDS IDENTIFICATION

Appearance and Odor:

Form: Viscous liquid

Color: Milky

Odor: Aliphatic

Potential Health Effects:

Eye: May cause eye irritation with susceptible persons.

Skin: Slightly irritating.

Potential Physical/Chemical Effects: Spills produce extremely slippery surfaces.

3. COMPOSITION/INFORMATION ON INGREDIENTS

Identification: Anionic water-soluble polymer in emulsion.

Regulated Components: None.

4. FIRST AID MEASURES

Inhalation: Move to fresh air immediately. No hazards which require special first aid measures.

Skin contact: Wash off immediately with soap and plenty of water. Get medical attention if irritation develops and persists.

Eye contact: Rinse thoroughly with plenty of water, also under the eyelids. Get medical attention if irritation develops and persists.

Ingestion: Rinse mouth with water. Do not induce vomiting. Get medical attention immediately.

5. FIRE-FIGHTING MEASURES

Suitable extinguishing media: Water. Water spray. Foam. Carbon dioxide (CO2). Dry powder.

Precautions: Spills produce extremely slippery surfaces.

Special protective equipment for firefighters: No special protective equipment required.

Flash point (°C): Does not flash.

Autoignition temperature (°C): Does not ignite.

Flash point : Not applicable.

Autoignition temperature (°C): Not applicable.

6. ACCIDENTAL RELEASE MEASURES

Personal precautions : No special precautions required. Wear adequate personal protective equipment (see Section 8 Exposure Controls/Personal Protection). Keep people away from spill/leak.

Environmental precautions : As with all chemical products, do not flush into surface water.

Methods for cleaning up : Do not flush with water. Dam up. Soak up with inert absorbent material. If liquid has been spilled in large quantities, clean up promptly by scoop or vacuum. Keep is suitable and closed containers for disposal. After cleaning, flush away traces with water.

7. HANDLING AND STORAGE

Handling

Safe handling advice : Avoid contact with skin and eyes. When preparing the working solutions ensure there is adequate ventilation. When using do not smoke.

Storage

Keep in a cool, dry place (0 - 30 °C). Keep away from heat and sources of ignition. Freezing will affect the physical condition and may damage the material.

8. EXPOSURE CONTROLS / PERSONAL PROTECTION

Engineering measures

Use local exhaust if misting occurs. Natural ventilation is adequate in absence of mists.

Personal protective equipment

Respiratory protection : Not required ; except in case of aerosol formation

Hand protection : PVC or other plastic material gloves

Eye protection : Safety glasses with side-shields. Do not wear contact lenses where this product is used.

Skin and body protection : Chemical resistant apron or protective suit if splashing or repeated contact with solution is likely.

Hygiene measures
Handle in accordance with good industrial hygiene and safety practice. When using do not eat, drink or smoke. Wash hands before breaks and at the end of workday.

9. PHYSICAL AND CHEMICAL PROPERTIES

Form : Viscous liquid

Color : Milky

Odor : Aliphatic

pH: 6 - 8 @ 5 g/l

Specific Gravity: 1.05

Melting point/range : Not applicable

Flash point : Not applicable

Autoignition temperature (°C): Not applicable

Vapor pressure (mm Hg): 0,002 @ 20°C

Bulk viscosity (cps): 1200

Kinematic viscosity @ 40°C (mm²/s): >>20.5

10. STABILITY AND REACTIVITY

Stability : Hazardous polymerization does not occur. Stable.

Materials to avoid : Oxidizing agents may cause exothermic reactions.

Hazardous decomposition products : Thermal decomposition may produce. Nitrogen oxides (NOx). Carbon oxides (COx).

11. TOXICOLOGICAL INFORMATION

Acute toxicity

Oral : LD50/oral/rat > 5000 mg/kg.
Dermal : LD50/dermal/rat > 5000 mg/kg.

Inhalation : The product is not expected to be toxic by inhalation.

Irritation

Skin : Slightly irritating

Eyes : May cause eye irritation with susceptible persons

Sensitization :

Not sensitizing.

Chronic toxicity :
No Chronic effects

12. ECOLOGICAL INFORMATION

Aquatic toxicity

Toxicity to fish : LC50/Danio rerio (Zebra fish)/96 hours > 100 mg/L (OECD 203)./96 hours > 100 mg/l, (OECD 203).

Toxicity to daphnia : EC50/Daphnia magna (Water flea)/48 hours > 100 mg/L (OECD 202).

Toxicity to algae : EC50/Scenedesmus subspicatus (Green algae)/72 hours > 100 mg/L (OECD 201).

Persistence and degradability : Not readily biodegradable.

Hydrolysis : Does not hydrolyze.

Terra Novo, Inc.
EarthGuard

MATERIAL SAFETY DATA

Revision Date: 10/3/2012

13. DISPOSAL CONSIDERATIONS

Disposal : Dispose of in accordance with local, state and federal regulations.

Container : Rinse empty containers with water and use the rinse water to prepare the working solution. Can be landfilled or incinerated, when in compliance with local, state and federal regulations.

14. TRANSPORT INFORMATION

DOT

Remarks : Not classified as dangerous in the meaning of DOT regulations.

IMDG/IMO

Remarks : Not classified as dangerous in the meaning of IMO/IMDG regulations.

ICAO/IATA

Remarks : Not classified as dangerous in the meaning of ICAO/IATA regulations

15. REGULATORY INFORMATION

US SARA Reporting Requirements:

SARA (Section 311/312) hazard class: Not concerned.

International Inventories **USA (TSCA):** All components of this product are either listed on the inventory or are exempt from listing.

Canada (DSL) : All components of this product are either listed on the inventory or are exempt from listing.

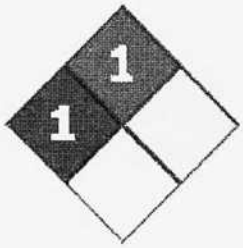
Terra Novo, Inc.
EarthGuard

MATERIAL SAFETY DATA

Revision Date: 10/3/2012

16. OTHER INFORMATION

NFPA and HMIS Ratings :



NFPA :
Health : 1 Flammability : 1 Instability : 0
HMIS :
Health : 1 Flammability : 1 Physical Hazard : 0 **MSDS was prepared in accordance with the following :**

ISO 11014-1: Material Safety Data Sheet for Chemical Products ANSI Z400.1-2004; Material Safety Data Sheets - Preparation

Contact : 661.587.5716

The data in this Material Data Sheet relates only to the specific material designated herein and does not relate to use in combination with any other material or in any process. This information is based upon technical information believed to be reliable. It is subject to revision as additional knowledge and experience is gained.

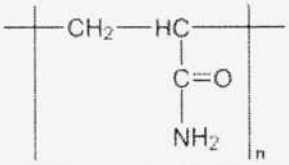
WIKIPEDIA

Page 80

Polyacrylamide

Polyacrylamide (IUPAC **poly(2-propenamide)** or **poly(1-carbamoylethylene)**, abbreviated as PAM) is a polymer ($-CH_2CHCONH_2-$) formed from acrylamide subunits. It can be synthesized as a simple linear-chain structure or cross-linked, typically using *N*,*N*'-methylenebisacrylamide. In the cross-linked form, the possibility of the monomer being present is reduced even further. It is highly water-absorbent, forming a soft gel when hydrated, used in such applications as polyacrylamide gel electrophoresis, and can also be called ghost crystals when cross-linked, and in manufacturing soft contact lenses. In the straight-chain form, it is also used as a thickener and suspending agent. More recently, it has been used as a subdermal filler for aesthetic facial surgery (see Aquamid).

Polyacrylamide



Names	
IUPAC name	poly(2-prop-enamide)
Identifiers	
CAS Number	9003-05-8 (http://www.commonchemistry.org/ChemicalDetail.aspx?ref=9003-05-8) ✓
ChemSpider	none
ECHA InfoCard	100.118.050 (https://echa.europa.eu/substance-information/-/substanceinfo/100.118.050)
UNII	5D6TC4BRWV (https://fdasis.nlm.nih.gov/srs/srsdirect.jsp?regno=5D6TC4BRWV) (1500 MW) ✗
Properties	
Chemical formula	$(C_3H_5NO)_n$

Except where otherwise noted, data are given for materials in their standard state (at 25 °C [77 °F], 100 kPa).

✗ verify (what is ✓✗ ?)

Infobox references

Contents

Uses of polyacrylamide

One of the largest uses for polyacrylamide is to flocculate solids in a liquid. This process applies to water treatment, and processes like paper making and screen printing. Polyacrylamide can be supplied in a powder or liquid form, with the liquid form being subcategorized as solution and emulsion polymer. Even though these products are often called 'polyacrylamide', many are actually copolymers of acrylamide and one or more other chemical species, such as an acrylic acid or a salt thereof. The main consequence of this is to give the 'modified'

Page 80A

polymer a particular ionic character.

Another common use of polyacrylamide and its derivatives is in subsurface applications such as Enhanced Oil Recovery. High viscosity aqueous solutions can be generated with low concentrations of polyacrylamide polymers, and these can be injected to improve the economics of conventional waterflooding.

The linear soil conditioning form was developed in the 1950s by Monsanto Company and was marketed under the trade name Krilium. The soil conditioning technology was presented at a symposium on "Improvement of Soil Structure" held in Philadelphia, Pennsylvania on December 29, 1951. The technology was strongly documented and was published in the June 1952 issue of the journal *Soil Science*, volume 73, June 1952 that was dedicated to polymeric soil conditioners.

The original formulation of Krilium was difficult to use because it contained calcium which cross-linked the linear polymer under field conditions. Even with a strong marketing campaign, Krilium was abandoned by Monsanto.

After 34 years, the journal *Soil Science* wanted to update the soil conditioning technology and published another dedicated issue on polymeric soil conditioner and especially linear, water-soluble, anionic polyacrylamide in the May 1986 issue, volume 141, issue number 5.

The Foreword, written by Arthur Wallace from UCLA and Sheldon D. Nelson from BYU stated in part:

The new water-soluble soil conditioners may, if used according to established procedures

1. increase pore space in soils containing clay
2. increase water infiltration into soils containing clay
3. prevent soil crusting
4. stop erosion and water runoff
5. make friable soil that is easy to cultivate
6. make soil dry quicker after rain or irrigation, so that the soil can be worked sooner

Consequently, these translate into

1. stronger, larger plants with more extensive root system
2. earlier seed emergence and crop maturity
3. more efficient water utilization
4. easier weed removal
5. more response to fertilizers and to new crop varieties
6. less plant diseases related to poor soil aeration
7. decreased energy requirement for tillage

The cross-linked form which retains water is often used for horticultural and agricultural under trade names such as Broadleaf P4, Swell-Gel, and so on.

The anionic form of linear, water soluble polyacrylamide is frequently used as a soil conditioner on farm land and construction sites for erosion control, in order to protect the water quality of nearby rivers and streams.[1]

The polymer is also used to make Gro-Beast toys, which expand when placed in water, such as the Test Tube Aliens. Similarly, the absorbent properties of one of its copolymers can be utilized as an additive in body-powder.

The ionic form of polyacrylamide has found an important role in the potable water treatment industry. Trivalent

Page 80B

metal salts, like ferric chloride and aluminum chloride, are bridged by the long polymer chains of polyacrylamide. This results in significant enhancement of the flocculation rate. This allows water treatment plants to greatly improve the removal of total organic content (TOC) from raw water.

Polyacrylamide is also often used in molecular biology applications as a medium for electrophoresis of proteins and nucleic acids in a technique known as PAGE.

It was also used in the synthesis of the first Boger fluid.

Soil conditioner

The primary functions of polyacrylamide soil conditioners are to increase soil tilth, aeration, and porosity and reduce compaction, dustiness and water run-off. Secondary functions are to increase plant vigor, color, appearance, rooting depth and emergence of seeds while decreasing water requirements, diseases, erosion and maintenance expenses. FC 2712 is used for this purpose.

Stability

In dilute aqueous solution, such as is commonly used for Enhanced Oil Recovery applications, polyacrylamide polymers are susceptible to chemical, thermal, and mechanical degradation. Chemical degradation occurs when the labile amide moiety hydrolyzes at elevated temperature or pH, resulting in the evolution of ammonia and a remaining carboxyl group. Thus, the degree of anionicity of the molecule increases. Thermal degradation of the vinyl backbone can occur through several possible radical mechanisms, including the autooxidation of small amounts of iron and reactions between oxygen and residual impurities from polymerization at elevated temperature. Mechanical degradation can also be an issue at the high shear rates experienced in the near-wellbore region.

Environmental effects

Concerns have been raised that polyacrylamide used in agriculture may contaminate food with acrylamide, a known neurotoxin and carcinogen[2]. While polyacrylamide itself is relatively non-toxic, it is known that commercially available polyacrylamide contains minute residual amounts of acrylamide remaining from its production, usually less than 0.05% w/w.[3]

Additionally, there are concerns that polyacrylamide may de-polymerise to form acrylamide. In a study conducted in 2003 at the Central Science Laboratory in Sand Hutton, England, polyacrylamide was treated similarly as food during cooking. It was shown that these conditions do not cause polyacrylamide to de-polymerise significantly.[4]

In a study conducted in 1997 at Kansas State University, the effect of environmental conditions on polyacrylamide were tested, and it was shown that degradation of polyacrylamide under certain conditions can cause the release of acrylamide.[5] The experimental design of this study as well as its results and their interpretation have been questioned,[6][7] and a 1999 study by the Nalco Chemical Company did not replicate the results.[8]

See also

Page 81

Chemical Degradation of Polyacrylamide Polymers Under Alkaline Conditions

Authors	David Levitt (Total Petrochemicals) | Gary Arnold Pope (U. of Texas at Austin) | Stephane Jouenne

DOI	https://doi.org/10.2118/129879-MS
Document ID	SPE-129879-MS
Publisher	Society of Petroleum Engineers
Source	SPE Improved Oil Recovery Symposium, 24-28 April, Tulsa, Oklahoma, USA
Publication Date	2010

Abstract

Hydrolysis of polyacrylamide-based polymers is rapid and extensive under the alkaline conditions typical of alkaline-surfactant-polymer (ASP) flooding. Even at room temperature, significant hydrolysis occurs within one to two months in the presence of sodium carbonate. While this implies that polymers used in ASP floods will rapidly become susceptible to precipitation with divalent cations, in most cases the alkali present will be the most sensitive component to precipitation so this may be a moot point. Also, autoretarding kinetics under alkaline conditions limit hydrolysis at 100 °C whereas complete hydrolysis occurs under neutral conditions. Furthermore, in-situ hydrolysis of initially unhydrolyzed polyacrylamide is proposed as a promising strategy for ASP floods since the injectivity of the unhyrolyzed polyacrylamide will be greater than hydrolyzed polyacrylamide due to its lower initial viscosity. The lower initial viscosity is not a disadvantage since once it has been hydrolyzed in-situ, its viscosity will increase.

Introduction

In this paper we will preserve the terminology of Muller (1981a, 1981b) in referring to the continued hydrolysis of partially hydrolyzed polyacrylamides (HPAM), which results in their sensitivity to calcium, as "chemical degradation,?? which is not to be confused with cleavage of the acrylic backbone by radical mechanisms, which Muller refers to as "thermal degradation.?? The distinction is a useful one, as the two

Page 8 | A

phenomena can largely be addressed separately in order to decouple their often competing effects on viscosity, as is done herein along with a companion paper (Levitt et al., 2010).

Early Work Concerning Chemical Instability of Polyacrylamide. The hydrolysis of polyacrylamide (PAM) and partially hydrolyzed polyacrylamide (HPAM) polymers at elevated temperatures was noted by Muller et al., (1980, 1981a, 1981b) and Shupe (1981) due to the increase of viscosity, change in pH, and evolution of ammonia observed in PAM solutions aged at elevated temperature, as well as precipitation with divalent cations. Muller et al. performed an early and in-depth analysis of the change in viscosity and conformational properties of HPAM with degree of hydrolysis (t) ranging from 0 to 0.49 in the presence of various amounts of NaCl, MgCl2, and CaCl2. They found that precipitation occurred when t exceeded about 0.3, but that this depended on the charge density (at), the product of t and the degree of ionization (a), which depends on the pH.

Davidson and Metzner (1982) noted the precipitation of HPAM when aged at 90 C in the presence of calcium and magnesium and determined that this was due to further hydrolysis of the polymer followed by the precipitation of an insoluble salt. After further investigation, it was determined that 70 C was the temperature at which precipitation became an issue in seawater for a time period of 200 days.

Zaitoun and Potie (1983) performed a detailed investigation of the precipitation phenomena between HPAM and calcium. They determined that precipitation can occur when the degree of hydrolysis (t) exceeds 0.35 at 30 C or 0.33 at 80 C. In the limiting conditions of t = 1, (i.e. complete hydrolysis, or poly(acrylic acid) (PAA)) precipitation occurs at the stoichiometric equivalence point, where 1 mol of Ca++ is present for every two moles of acrylate moiety. As degree of hydrolysis decreases, an increasing excess of calcium over stoichiometric equivalence is required before precipitation occurs, but this excess does not depend on polymer concentration. In this region, the precipitation is described in the terminology of Ikegami and Imai (1962) as a site fixation phenomenon. At lower t, below about t = 0.6, the precipitation phenomenon is theta type, resulting from poor solvation. In this region, the critical degree of hydrolysis is independent of polymer concentration and redissolution is observed at very high calcium concentrations.

File Size 342 KB **Number of Pages** 9

NOV 01 2019

Preliminary Infrared (IR) analysis:

An alkaline digestion of the material using a moderately strong (1-3M) solution of KOH was made. This appears to have been successful.

A characteristic pink color arises during this digestion process.

This approach simulates the alkaline degradation of polyacrylamides that is referenced in the literature.

The color of the solution can be neutralized by bringing the solution to a neutral pH.

IR peaks of absorption to consider are

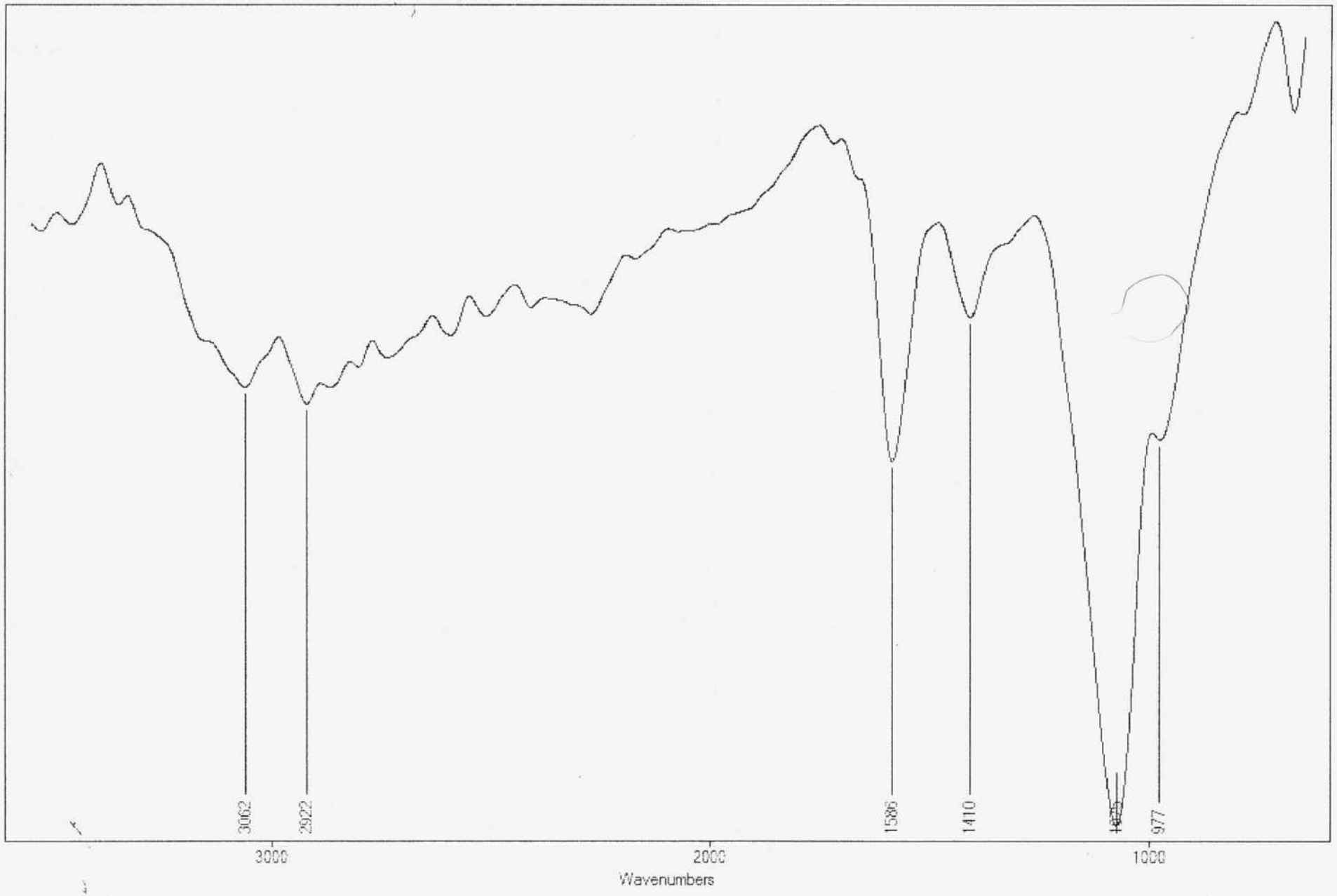
3062, 2922, 1586, 1410, 1075 & 977,

The method used is ATR placing a film Crystal ($KOH + HCl \Rightarrow H_2O + KCl$) of the neutralized solution on the plate.

Our primary absorption peaks of interest are:

1586
1410
& 1075

Earthguard KOH digestion - ATR - KCL Blank Removal - Average Nov 01 2018 - 03.spc: Synthetic spectrum by averaging. See audit trail for details.



printed on 11/01/2018 16:37:55

First question, do we have a Carbonyl?
Normal range is 1820-1600.
We have 1596. Investigate this further.

N=O Nitroso is given @ 1500-1600. (IR Pal)

Pavia gives Nitro groups @ 1600-1500
& 1390-1300
Reasonably close w/ second absorption @ 1410.

The case for the Carbonyl seems weaker @ this point and the case for the nitrogen group appears stronger.

For 1410, IR Pal gives S=O sulfates @ 1350-1450

It is known that polyacrylamides (PAMs) can degrade to nitrogen and carbon compounds. Carbon & nitrogen oxide forms have been mentioned in papers, as well as nitrates.

One study shows no monomers formed as a result of the degradation, but other sources indicate that it may occur.

Two points to make:
1. What is the structure of the monomer?

2. What are the degradation methods?

(2) There are several degradation methods. These include:

1. Thermal
2. Photolytic
3. Biological
4. Chemical
5. Mechanical

} The potential likelihood and effects from each of these methods will need to be considered in detail to assess the likelihood of monomer formation.

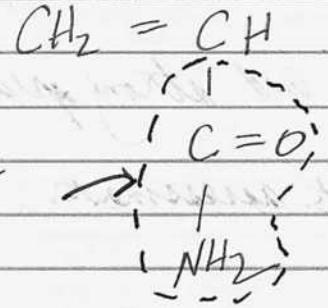
A highly recommended paper is:

"Chemical & Photolytic Degradation of Polyacrylamides used in Potable Water Treatment"

Peiyao Cheng - Univ of South Florida
2004 M.S. Thesis. 106 pp.

Available online in PDF format.

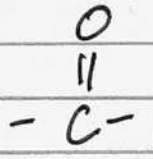
(1) The structure of the monomer is given as:



Acrylamide Structure

Critical functional groups to identify therefore are

1. Amine group
2. The Carbonyl group
3. The amide group.



Amide group

At this point, we do not see evidence of these Critical functional groups within the material that has been degraded (digested) IN THIS PARTICULAR MANNER (emphasized)

Other degradation methods are likely to produce different results.

Lets also look @ 1075 cm^{-1}.

Pavia gives two candidates that seem reasonable to consider

X			
1125	C=S	(1050 - 1200)	Thiocarbonyl
1110	R-O-R	(1070 - 1150)	Ether

Of these two, the C=S would seem to be more likely.

Parker (Applications of Infrared Spectroscopy) in his table

gives C=S @ ~1120 and strong peak.

This continues to be my best assessment.

At this time, I would postulate that we may be seeing degradation products:

1. N=O
2. S=O
3. C=S

At this point, this does not satisfy the monomer degradation hypothesis.

This may indeed occur, but additional degradation studies will be required to examine this.

This work postulates that nitrogen, sulfur and carbon compounds may result in the degradation process.

This is as far as this study can proceed @ this time. Electrochemistry techniques might be useful to further substantiate this result and to look for inorganics (eg metals) in the materials.

Nov 05 2018.

I will now look @ the second sample material received. The come from Mission Viejo in Aug of 2018.

There are two sample types. They are stated to come from a former pool that is now turned into a pond.

Sample 1: Appear to be pond scum.

After observation under the microscope @ ~ 3000 X I conclude that it is indeed "pond scum". Algae & protozoa comprise the sample, as most I can see. I see nothing unusual @ this time. It was stated to be biologically active w/ bubble formation. This would not surprise me. I have taken photographs. A protozoan - worm like - is the most common life form. It should measure ~ 20 microns long.

Sample 2 is more uncertain. It does appear to be a salt.

1. It dissolves steadily in water.
2. PH appears to be essentially neutral.
3. It is highly conductive in solution.

We already know therefore that we are almost certainly dealing w/ (an) ionic salt.

An ionic salt that is highly soluble (at least moderately so, and highly conductive.

I could try infrared to see if we can pick up anything there.

Electrochemistry is our best tool, however. It has been a while. I will start w/ AC Voltammetry again.

We must revisit and refamiliarize ourselves w/ the methods.

—

I begin again by seeking repeatability of results. Some work was done in VOL 21 of notes w/ AC voltammetry trials

—

I am seeing repeatability.
My first trial is @ $E_0 = -3V$ and $[-3, 3]$
Parameters are

$E_{equil} = 0$
$E_{begin} = -3V$
$E_{end} = 3V$
$E_{step} = .02V$
$E_{ac} = 0.05V$
Scan rate = 0.1 V/s
Frequency = 100 Hz

and now ☑ Measure DC Current!

I now highly stable and convergent results!

Let's start by looking @ the zero crossings:

ACV'
[-3, 3]

-.91V
.004V
+.55V
+1.20V

ACV
[3, -3]

-2.62
-2.28
+.99V
+2.49V

Now look @ original curve peaks

ACV
[-3, 3]

-.93V
+1.19V

ACV
[3, -3]

-2.28
-2.66
+.97
+2.45

$\overline{X}$:

-0.92
+1.29

$\overline{X}$:

-2.64
-2.28
+.98
+2.47

Now let's look @ the redox means of combined ACV & ACV', forward and reverse scans

2.64 N_2(2.65) Mg (2.68) Na (2.71)

2.45 No common match

2.28 2.25 (H_2) SO_4 (2.12)

1.20 Cl (1.25) (Numerous) H_2O (1.22) SO_4 (1.12) Mn (1.19)

0.98 NO (.99) NO_3 (.96) Cl (.95) NO_3 (.94) .93 (SO_4)

0.92 SO_4 (.93) NO_3 (.94)

Strong Candidates as shown:

$MgCl_2$ Chemical tests have been performed.
NaCl Chlorine test is negative
$MgSO_4$ Sulfate test is positive

We now need to test for Mg.

Now we test for the Mg^{+2} ion.

Any alkali will form a white precipitate with either

~~Ammonia~~ Aluminum
Calcium
or Magnesium ions.

Aluminum dissolves (ie, the precipitate of aluminum hydroxide) in excess NaOH. (We are using KOH).

The did not happen, in fact, the more alkali added the more the precipitate formed.
In addition, aluminum is not on our story candidate list.

Ca & Mg ions remain as candidates.
Flame test may be able to resolve the difference.

However, we note that Ca is also not on our candidate list.

$MgSO_4$ is definitely our leading contender. Physical appearance of the salt (ie, epsom salts) also match the conclusion as it is familiar w/ epsom salts.

In addition, we are also quite familiar w/ $MgSO_4$ crystals under the microscope.

So we have at least 2 more ways to separate the difference.

Flame Test:

Calcium: - Orange - Red
Magnesium: Bright White

I have now completed the flame tests, using a reference of $CaCl_2$ solution. Indeed a red color does show up in a flame test of the reference solution.

Our sample solution fails this flame test, no red is visible. White is also not visible. However this may simply be due to light condition.

Our conclusion, therefore, is that we are dealing with an environmental sample in a pool a pond composed predominantly of magnesium sulfate.

The microscope will be used to finalize the conclusion w/ direct observation of a crystal evaporation slide.

Ok, that is I am thrown for a little loop here.

The crystals formed, in the case, by heating are CUBIC shaped.

This was not expected from past experience.

Mg sulfate form a hexagonal crystal?

The melting point is 1124°C so that is not going to be helpful.

$MgSO_4$ is an orthorhombic crystalline structure.

Ok, here we go. Orthorhombic is indeed based upon a cubic structure however $a \neq b \neq c$.
ie it is stretched differently along each axis.

It does, therefore results a rectangular prism.

The down fit the observation as you notice the crystals are all different sizes (not like NaCl) and they are rectangular in shape. Photo taken

Let me a solution that cools slowly and compare this.

OK, when I let a slide air dry instead of drying w/ forced air, the crystalline structure are much more varied, and portions of the slide conform to previous observations of epsom salt crystal structure.

The conclusion does remain as a primarily magnesium sulfate compound.

I have run an additional chemical test for verification. It is given that washing soda and epsom salts, when mixed together, form a white precipitate.

When I add washing soda to my sample, I get the same white precipitate forming.

$$MgSO_4 + \overset{Na_2CO_3}{\cancel{CaCO_3}} \rightarrow MgCO_3 + Na_2SO_4$$

X The conclusion is satisfied by:
1. Electrochemistry - Candidate identified
2. Solute test
3. Flame Test
4. Microscopy
5. Sodium Carbonate test

The primary question is how did the epsom salt form in a pool or pond?

An enquiry will need to be sent to the citizen to see if more can be learned about the situation.

Nov 07 2018

I have started two sets of cultures today.

1) The first is Protozoa
   1. Water
   2. Hay infusion (Home Science Tools)
   2. Cat food pellet as nutrient source

2) HEPA filter cultures

tsp	1. sugar	1
½ tsp	2. $FeSO_4$	2
~0.2 gms	3. Potato flakes	3
~.02 gms	4. Salt (pink)	4
		5. 3 drops H_2O_2

~100 ml H_2O

Green Tag Orange Tag

In addition, we have the aquarium started w/
   1. Water
   2. Air
   3. Sponge filter

I have now added
   1. Protozoa hay infusion source
   2. Cat food pellets as nutrient source

Examination of the commercial HEPA filter shows the material collected to be

1. Hair fibre
2. Particulate matter
3. The infamous "environmental filament"

It seems to be quite common →

It is astounding how frequent the environmental filament" is appearing in the material.

A small sample of the filtered material is used as the basis for culture now under incubation @ 85°F.

And the blackworms did arrive.
I have started a ~ 1 gallon aquarium to see if I can create a good culture that will support blackworm growth.

In addition, a 5 gallon aquarium is on order.

Note, after approx 6 hrs of incubation of the HEPA filter cultures, the peroxide culture (orange) is very clear.

The non-peroxide culture is cloudy.

This indicates potentially more growth in the non-peroxide culture.

Disregard for now

Nov 09 2018

~~Important Culture Development & Observation~~

DO NOT DISREGARD! SEE NOTES FOR NOV 11, 2018

Page 99

I witness the most amazing culture growth and transition in the shortest time period that I can recall.

The culture under study is the "green coded version", i.e., without the H_2O_2 added.

Sugar, Himalayan salt, $FeSO_4$ & Potato flakes incubated @ ~ 80-85°F.

The source for the culture is the collected material from the commercial grade HEPA filter.

In 24 hrs Chain formation of the CDB could be seen to be taking place.

broad

Now, in 48 hrs, massive Chains are now forming ~100 - 200 CDB's wide, Coalescing into Mycoid & large Chain - filament form.

The growth pattern is indeed remarkable.

Microphotographs have been taken @ ~ 600x and ~ 3000x (3000x).

An amazing and rapid growth pattern is underway.

The growth medium appears highly favorable to rapid & more complex growth.

Mature filaments are also visible in the culture, however it is uncertain whether these simply originate from the originally collected material.

Time will settle the question easy enough as the number of such filaments in the microscope slide viewing area is relatively few at this time.

The rapid-coalescing growth is dominant and extensive of the 48 hr culture.

Nov 10 2018

For whatever reason, the microscopic examination of the HEPA culture 24 HRS later does not reproduce the result of the prior previous day.

There is no pattern of coalescing growth apparent in the observations today.

For the time being we place the observations of Nov 09 2018 ON HOLD and we let the culture continue to develop.

Possible explanation: Delivery from the pipette onto the slide produced a striated presentation on the slide??

At this point, ~72 hrs into the culture, there is no known and obvious growth process taking place. There is no apparent filament network ~~dev~~ developing @ this time.

Observed @ ~ 800X.

Now for the lint roll culture - 24hrs

No growth pattern visible.

In both samples, we are primarily looking at iron oxide this far.

However, what we do know is that both the HEPA filter and the lint roll sample both clearly show very high numbers of the "environmental filament" structure.

The lint roll sample comes from a home environment that reports serious health symptoms from Magellons across the entire family - married couple and kids.

The samples are well documented by a data analyst of 20 years experience.

The goal here is to attempt to develop cultures from more readily available environmental samples, such as HEPA filters, for example.

Both citizens have delivered HEPA filters. The commercial filter examined under the microscope shows a massive collection of the environmental ("Magellon") filament type within it. also"

The lint roll shows the same.

Both samples also show normal hair present - they are both a mixed sample.

Our next move, while the initial cultures develop, is to attempt a microwave digestion of the HEPA filter sample. The filter has collected large amounts of material to work with.

—

The protozoa culture is succeeding, after 48 hrs. The species appears, however, to very small. It would be easier to document toxicology studies if we can get a larger species to develop.

Ok, this is working well. The slide must simply be prepared fresh.

The culture consists of
1. Water
2. Hay infusion
3. Cat food pellet.

After 48 hrs, there is a film being produced on the surface of the water. The sample is dense w/ small protozoa.

800x gives a better presentation than 3000x does.

The heat on the slide, & light, does reduce their motility, so comparisons will need to be made during identical time frames.

But this can be done.

We can use the culture, w/ the film developers, as an a feeder culture.

You can increase the mag. rate of the video, a crop it, but I am not sure it enhances the perception of motion to any real benefit.

The protozoa @ the bottom of the culture, ie, at the Cat food, seem to be even smaller.

We can work w/ what we have and see if other species develop.

I have set up another culture. This uses a microwave NaOH digestion of the Commercial HEPA air filter material. Composition:

1. ~200 ml H_2O
2. Microwave digested 30 min low power NaOH HEPA collected material (hair - particulate matter - env. filaments)
3. Teaspoon sugar
4. Min. pinch pink salt
5. 1/4 tsp potato flakes

Incubate @ 85° F.

Nov 11 2018

Commercial HEPA culture observation: ~4 days old

There may be an increase in the density of the "env. filament" network developing. Fully developed filaments seem to dominate the culture alloy w/ the iron oxide formation.

What is unusual is that there are no, or no significant presence of normal hair fibers, the flattened structure + twists characteristic of the env. filament are identifiable as characteristic.

It is possible that the increase is also detectable by eye upon inspection of the bottom layer in the culture jar itself, with the proper background lighting.

A dozen unique filaments have been easily identified w/in a couple of pipette drops taken from the bottom layer culture.

(OK) Now we have a VERY intriguing situation.

For the first time we are now looking at the "orange coded" culture set up on Nov 04 2018

What we all do the same exact linear coalescing structures (large in size, ~200-400 microns long, ~ 50 microns wide)

that we saw in the "green coded" culture (i.e, no H_2O_2) first observed on Nov 9.

Therefore the notes of NOV 09 2010 are not to be ignored or discarded.

We now have an unexplained repeatable event a formation that is taking place.

By all appearances, the H_2O_2 modified culture is only lacking in time for the identical formation that took place earlier in the non-H_2O_2 culture.

This is not a "mistake" in slide preparation it does not seem. This will now be watched very closely in future culture trials.

In addition, in a manner identical to that of the "green" culture, filaments at this stage of development exist within the orange culture @ this time, but they do not dominate it.

We may have repeatable stages of development that are taking place within these experimental HEPA culture trials.

Time will tell. This is NOT just random iron oxide formation.

Our next set of cultures are only 1 day old.

1.) The first is a culture based upon microwave alkaline digestion of the HEPA material. This is done to separate the solids (eg hair, etc, all solids) from the collected material. The digested material is consequently run through a coffee filter so that a uniform solution (devoid of solids) is the base of the culture. This is therefore a culture of more pure form also anticipated to contain a higher composition of Fe CDB. Microwave settings used were at the lowest possible for 30 min in a strongly alkaline solution (KOH).

The culture therefore consists of:
1. ~200 ml H_2O
2. Filtered, digested HEPA collection (now in solution) 5 drops used in culture.
3. Teaspoon sugar
4. 1/2 tsp. $FeSO_4$
5. 1/4 tsp. Potato Flakes

Incubation @ 85°F.

Observation shows that:

We already have something happening within 24 hrs on the culture.

The culture is showing a slight formation of material on the surface of the culture.

Close observation, even by eye, reveals that it is of stranded form.

Furthermore, all of the strand formations are lining up in the same direction on the surface of the liquid, or parallel to one another in formation.

Further observation over time will examine this orientation, however, at first glance we may be dealing with formations that are aligning with magnetic north,

similar to placing the paper clip on top of a cup of water to act as a magnetic compass.

I have done this survival hint myself some time ago.

Now, the next observation is under the scope @ ~800X.

Result: We are indeed dealing w/ a filament network that is forming w/ in 24 hrs using this digest/ferment method of culturing.

We may be in for a method of rapid & repeatable filament formation within a controlled culture development.

Time will tell.

Well, I can already tell.
I do indeed have it.

This is classic CDB filament formation.

This time, within a ~~dish~~ developed and controlled & repeatable culture medium, within a period of 24 hrs.

Also the source of the culture is now a readily available environmental sample ie, HEPA filter.

Culturing is no longer dependent upon a human biological sample, which also has been accomplished by numerous different methods over the years

I now have a method highly controllable method of producing the CDB (ie, "Morellons" filament network in a very short time period.

Microscopic images have been collected. 3000x, vs 600x is much more appropriate for examination here and the detailed CDB-filament structure is evident.

This is another momentous event of discovery and method here today

Certain growth patterns here have never been witnessed before in such detail and with skyperhi image collection.

The development of a star-like network formation is seen for the first time here today. This is a radial form of the filament network. It is a very effective and rapid growth pattern for reproduction.

The method is monumental in its ability to produce controlled, rapid & pure growth of the filament network.

The filament network can now be studied to the level necessary to understand its growth & biochemistry, and eventually how it affects the biology of other living organisms, including humans.

This is indeed a monumental day.

The digested source material in solution, and the culture medium used is critical to the progress that has been made and will be made in the future.

Nov 12 2018

An email recording the events of yesterday's observations has been sent to:

1. The CI email address.
2. A port particular group of lawyers

for record & posterity purposes.

—

Today, I resume observation of the microwave dissected HEPA filter culture.

The observations of Nov 12 hold as stated.

—

Additional comments and observations:

1. I have examined both surface and bottom layers of the culture. Identical formations occur in both layers.

2. There is absolutely no doubt that the cocci form of the CDB aligns itself to form the filament networks. This was first recorded in the "Growth Progressions" research paper.

3. 3000x is sufficient to reveal the structural detail of formations.

4. Thus far there are the following structural forms visible:

1. The individual CDB (huge numbers)

2. The formation of filaments often with encased CDB within (also very numerous)

3. The formation of a radial filament network where growth appears to occur rapidly

4 Isolated ring structures (a) of the CDB, approximately the size of a red blood cell, have formed in the clotform layer of the digested HEPA culture. Four examples have been captured on microphotographs.

Additional cultures of the same type are now to be created to increase production of these CDB-filament networks.

5 additional cultures have now been started.
1. Digested HEPA solution ~ 6 drops each
2. ~200 ml H_2O each
3. Tsp sugar
4. 1/2 tsp $FeSO_4$
5. 1/4 tsp potato flakes
6. Incubation ~ 95°F

The next thing we learn is that we now have a highly successful paramecium culture w/in the newly created aquarium

The aquarium (now 1 gallon), soon to be 5 gallon, currently contains ~ 12 blackworms and now paramecium. There are some small pond shrimp / daphnia but I am not sure if the will survive.

Snails & plants will be introduced into the aquarium along w/ an increase in size to ~ 5 gallons.

The paramecium, snails & worms are slated for toxicology testing as the habitat is successful.

—

Next we look @ the "green culture" (ie HEPA raw material, no H_2O_2)

@ 800x the culture is once again dominated by the large ribbon-shaped formations in the culture.

The material comes from the bottom layer.

It is presumed the bottom material consists largely of iron oxides but the proposal will be scrutinized as the project proceeds.

It is a most curious development as there is no reason to think that iron oxides will result in such structure. It is possible that these are CDB formations.

Time will tell.

The slide example, from the bottom layers of the culture shows few natural filaments as we observed yesterday.

Now we will start examining these structures @ 300X.

I will reserve judgement until much time passes, however I am prepared for the finding that we are dealing w/ massive structural collection and formations from the CDB.

I am also seeing the presence of the purple color and circular structure that capture so much of my interest during skin and plant examinations over the last year.

We will now, for the first time, look @ the culture @ a level of 8000x.

We do indeed have the circular structures forming that are ~ 6-8 microns in diameter.

Indeed also the CDB can be seen within the interior of the circular structures, exactly as was witnessed w/ blood cell examinations many years ago.

We also see dense circular concentrations appearing in a oval-circular structure that is ~ 25 microns in diameter.

Somehow I suspect these ribbon structures, although massive in size, are indeed composed of CDB collections.

This will be quite amazing in its own right, considering the simplicity of the culture method, in general.

We are definitely dealing w/ organized structure and growth here, not random collections of iron oxide.

Two major cultural developments are now being witnessed.

Nov 14 2018

Went to Farmington NM last night to retrieve the osmometer. Looks like a success story is in order there.

Returning to culture observations.
Starting w/ the "green code" culture of Nov 07.

We now have a massive filament dense growth network that has taken place @ the bottom of the culture. It is dense enough that it has formed a matted layer.

A wispy filament nature can be observed by eye. The microscope @ 800x - 3000x, however, reveals the extent of the growth. It is massive.

The means that the ribbon structures observed prior have now transformed into the filament network.

This has all occurred w/in 1 week of time.

There now will be ample material to study any way that you need to, including sequencing.

DNA extraction would also be a very substantial problem from this dominant filament form.

The network is very much rust colored, ie the iron has now been oxidized it would appear, and incorporated into the growth.

Time lapse photography would also be a fascinating to pic here.

This represents a major achievement in the culture process.

The culture has again been photographed under the scope @ 800-3000X.

You can now use this culture as a jump start culture to the next one. You should now have an ongoing filament supply. Very good.

Ok, now on to to digested HEPA culture:

Very interesting results. We have a different form of growth taking place. The ribbon structure are no longer in place here. The developing filament network is also not in place.

What we do have now is:

1. An apparent break up of the rebelion like structures into what random appearing chunks or blocks.

2. The transformation of these blocks into globular or oval structures, almost like very large cells. These "cells" or units are quite large in size, estimate roughly up to ~100 microns across.

These structures are large, and 800× is more than sufficient to show their overall shape.

In addition, the repeatedly observed "deep purple" color is coming into being w/in these structures.

There also is no surface filament layer in the culture.

The culture has undergone a significant transformation from its original form that was developing a filament network.

Our question ahead is, what will these large "cell-like" structures become?

OK, it would appear when we boost up to 8000x that we are able to see the early stages of massive filament networks developing within the "cell-like" units.

It appear as they it may headed on a course of action similar or identical to that previously just observed w/in the green code culture.

Clearly we have filament structures forming within the cells, and it looks to become a very dense network.

Time will tell.

The cultures are very pure w/ little to no contamination.

I have collected pond plants & foliage today from Monticello Lake, which should be more properly called Monticello Pond.

I have an invertebrate - protozoa aquarium project in place which is to be used as a source for toxicology studies in the future.

Protozoa, blackworms & snails are being cultured for the project.

Paramecium are the target protozoa; some success has already been achieved here.

W.r.t. cultures, I have expanded the scale of the culture project considerably (again). Because of the remarkable success of the HEPA pellet cultures both raw and digested, I have expanded the scale of the raw HEPA pellet culture project.

What is different here is that the HEPA pellet material will now not be used. This will eliminate the question of external contaminants (eg hair) and particulates.

The seed for the culture is a rather small portion of the matted filament network that developed quite rapidly in the "green" coded culture, ie

~200 ml H_2O
HEPA collected material (mixed)
based upon microscopy examination.
~tsp sugar
~1/2 tsp $FeSO_4$
~1/8 tsp potato flakes
~pinch Himalayan salt.

My scale of culturing is now:

2 glass casserole dishes (cake pans) 10" x 10" x 2"
w/ ~1000 ml each.
~5 tsp sugar
~2 1/2 tsp $FeSO_4$
~5/8 tsp potato flakes
~1/8 tsp Himalayan salt

} for each glass container.

The hope is to collect a significant amount of filament material for analysis & investigation. This would eliminate the supply problem and remove the unpredictable aspect of filament stage growth in previous cultures.

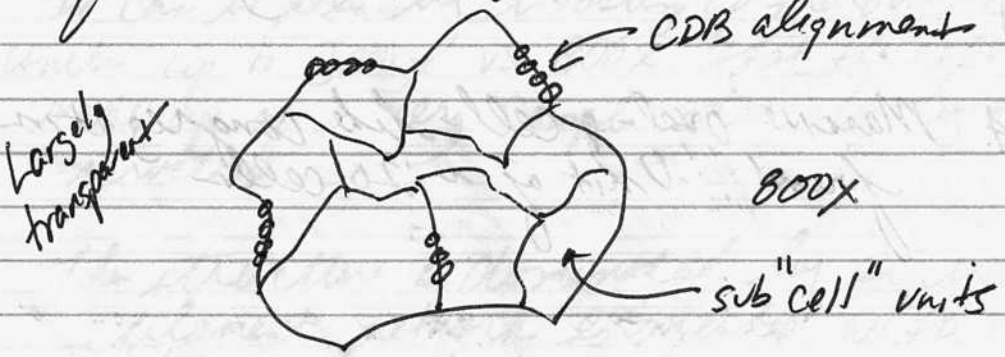
Estimated time is 1 week.

The next order of business is to now observe the digested HFPA culture and to look for any change:

The digested culture is largely the same. We still have the large "cellular" formations in place.

There is one change, however. In addition to the "cellular" formations, there is another style of organization taking place.

These also are of a "cellular" nature but they are much more transparent in nature. Aligned CDB's often form the boundaries of the cell units.



Large formation (estimate ~300 microns diameter) composed of sub units (~50-100 microns) of occasional CDB alignments along boundaries of sub-units & ellerior boundary.

Nov 16 2018

Monitoring the 10" x 10" culture based upon raw HEPA collection : (24 hrs)

1. The oval structures are already starting to appear in the culture. We have the apparent Congregation of the CDB taking place as the iron oxide hypothesis does not hold up in the main. III (Count of 3)

2. I see appearance of a filament that appears newly formed. IIII (Count of 5)

3. We do not see the ribbon structures formed, except in possible early stage.

4. Massive oval-like cell-like congregation found. Unit of ~ 20 cells.

Next we observe the bottom layer of the digested HEPA filter culture.

There is a thin bottom layer, film like. It contains one filament strand/wire network.

The structures described yesterday (CDB boundaries - cell conglomerates)

match quite well photos in the current American Laboratory trade magazine in an article about "protein Conglomerates"!

This seems to be quite likely what we may be dealing with here.

It can be seen by boosting up the oval-cellular units up to 3000x vs 600x that the cell is made up of a very complicated biological structure.

The structure is dominated by an internal filament network enmeshed with the CDB throughout.

Analysis of such structures, biochemically speaking, is @ the heart of understanding of the Morgellons condition.

Both raw & digested cultures now produce filament - CDB networks w/ characteristics varying to some degree - ie, digestion vs raw.

Essentially we have a protein being formed within the digested culture.

The again defies any normal expectations.

Essentially we seem to now have 3-4 different protein formations, each without reproduction of the original organism except for the first extraction method

1. Extraction of biomolecules method (see patent application)

2. The "secreted protein - incubation method

3. Two different "cellular" units, one method from raw collected HEPA material and the other from digested HEPA filter material,

both placed into the same medium

Nov 19 2018

Monitoring the 10"x10" culture based upon RAW HEPA filter collection (4 days old)

There is complete success of the culture. A significant matted sections are now appearing on the bottom ~~of the culture~~ layer of the culture.

Size of these sections are ~ 1-2" across.

The matted sections indicate that a filament network has been formed, I am presuming that non-matted sections (still the vast majority of the culture @ 4 days of age) are protein conglomerates.

I will run tests to the effect.

There is the first of more pure culture as it was a previous culture as the starter material there will be no limit to the amount of filament network that can be developed now.

Let's try to digest a portion of a matted network as well as an unmatted portion.

OK, the culture has been digested and an IR plot developed.

Shows what the closest match is from the database of 6000+ spectra?

The CDB secreted protein spectrum acquired in DEC 2017.

You can certainly say that we have a match.

This method of producing the protein takes approximately 4 days instead of the 2-3 weeks required for the secretion method.

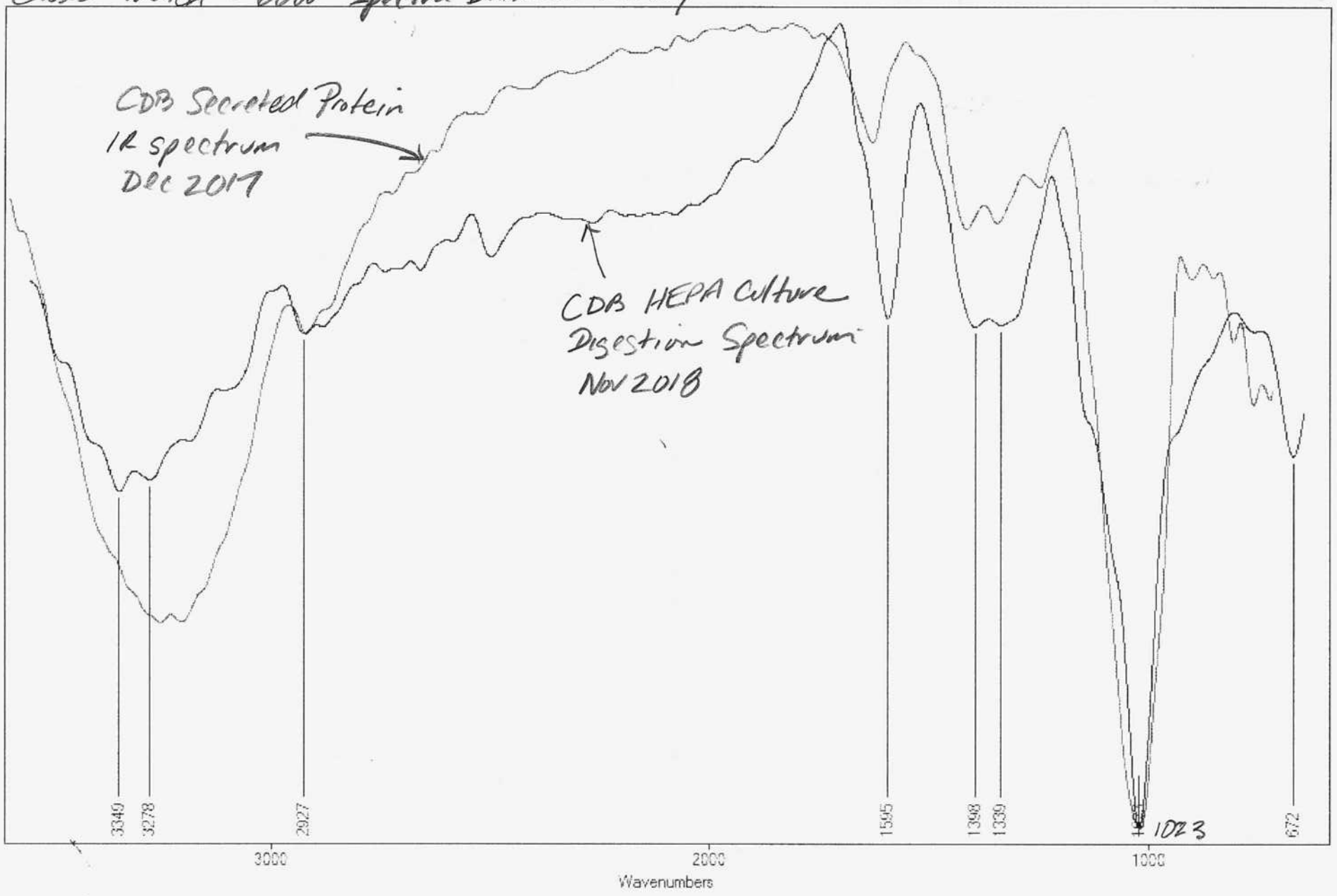
This again offers numerous advantages for laboratory procedures.

The form of the protein is difficult to digest or it is not water soluble. But the chemical composition must have many similarities to the secreted protein form.

Digested HEPA CDB Culture - IR Spectrum

CDB HEPA Culture Digested - Neutralized Nov 19 2018 - 01.spc: 11/19/2018 17:02:00 title

Closest match - 6000+ Spectra Database Library



printed on 11/19/2018 17:10:32

Nov 20, 2018 Page 1/30

There are some important lab issues ahead of us.

The culture methods achieved are quite remarkable. The methods are productive and rapid. Three to four variations on CDB morphology are now accessible.

1. Coccus form - most primitive
2. Protein Conglomerate
3. Secreted Protein
4. Extensive filament network

Each of these is available through various culture methods, and each has their place in the various studies.

The ability to use HEPA filter source material is another breakthrough.

Needs & plans now include:

1. Run the Bradford test on
   1. The digested HEPA source material
   2. The digested protein conglomerate & filament network combined (Digestion is very difficult here).

   Both the digestion solution and the settled solids.

2. What is the state of sugar concentration in the culture?
   The polarmeter could be useful here.

3. Remember your highly sensitive protein test that you developed — this also can be very helpful here.

4. Can you extract DNA from the HEPA based culture? If so, raw HEPA source material.

5. Inhibiting the rapid culture growth is an intriguing prospect. The turnover time now will be much shorter. You have previously demonstrated the effectiveness of anti oxidants, ie, reducers, such as VIT C & NAC! I would like to explore the possibilities of ultrasound and electromagnetics in the yeast. You do have some preliminary work already established w/ electromagnetics. It seems to be easier to enhance growth than to reduce it thus far.

6. Continue to monitor cultures; they are quite rapid & dynamic now. Collect materials & consider drying & grinding to a fine powder for transport.

7. Blood sample & alternative HEPA filters are of interest.

8. The skin examination project is of interest.

Ok, two culture exhibition experiments have been started.

1. The first is Ultrasound energy @ 5MHz introduced into a HEPA Hard culture (seeded).

2. The second is a frequency sweep (popularly known as Rife) applied to a culture.

The plan: watch and observe for any difference in the growth pattern.

—

I want to recover my protein detection test that I developed as apparently it is quite sensitive.

Book 18 April 13 2017 has a reference - RH red dye

Book 17 April 03 2017 has a reference Red Dye #3 also

See note on Red Dye #3 on Apr 03 2017

Book 17 Mar 22 2017 Developed test referenced.

Red dye #3 (Carmoisine) appears to have produced very good results
Reagent developed:

3 ml H_2O
1 drop Conc. NaOH
1 drop 0.5 M $CuSO_4$
visible tartaric acid

[1 drop dye in 1 ml H_2O] diluted to 30 ml H_2O
then add 30 ul??? added to reagent

It has taken me a lot of work to reconstruct the reagent developed for protein detection

The last notes were on April 03 2007
about 8 pages in.

The reagent is constructed by

1. First taking the dye (either red dye #3 or RIT red dye) of 1 drop into ~ 1 ml of H_2O
Now take the first dilution of the red dye & then dilute it further to ~ 30 ml total H_2O
The red dye is then very dilute.

Now the reagent is formed with:
~ 3 ml H_2O
1 drop Conc. NaOH or KOH
1 drop 0.5 $CuSO_4$ #3!
50 30 ul of the dilute red dye above
= tartaric acid sufficient to clarify the reagent

Hold this as a control solution.

Now if you add 50ul of dilute powdered milk solution you should detect the color change.

Here is what we learned today.

The use of Red Dye #3 (food coloring dye) appears to be superior in the development of the reagent. The color change appears to be more noticeable.

The control solution has a more blue hue. The solution w/ the dilute protein added (50ul) appear to be a more greenish hue.

You have also increased the dilute red dye #3 added from formely 30 ul to 50ul.

RIT dye we may work but Red Dye #3 appears to be more repeatable and discernible.

The detection reagent will hence forth use Red Dye #3.

Now verify w/ VIS spectroscopy

Ok, we positively have a distinctive color shift by VIS spectrometry. This is indeed a very sensitive test for soluble protein detection.

The values are 664 nm $\overset{vs}{\leftrightarrow}$ 686 nm.

Very detectable and repeatable.
Steve & mixed up the tube sets & must repeat to determine the proper direction.

The original should be @ 664 nm (greenish blue)
The protein solution should be @ 686nm (bluish green)

Let's see.
The $CuSO_4$ must be completely dissolved w/ the tartaric acid before you proceed adding the proteins.

	Control	Milk	Δ
1	681nm	712nm.	+25nm
2	663nm	685nm	+22nm
$\bar{X}$=	615	699	+24

Ok, here is where we are at. The method will be to double the reagent volume, i.e. 6 ml instead of 3 ml. 1/2 of the reagent will be used as the control, the other 1/2 will be used to determine if a protein SHIFT towards the blue green portion of the spectrum occurs.

Having a stable reference control that is common to both samples along w/ the detection of a shift of ~ 25 nm is the key to protein ~~detect~~ detection here.

The dye indeed appear to be an excellent method of protein (soluble) detection only that has been developed.

Therefore the reagent recipe is now:

6 ml H_2O
2 drops Conc. NaOH or KOH (~10M)
2 drops @ 5 M $CuSO_4$
Tartaric acid (minimum level necessary to clarify the solution)
100 ul of dilute red dye #3

Red dye #3 dilution is in two stages. Actually, not required.

Simply use 1 drop red dye #3 in 30 ml H_2O. Then use 100 ul of that dilute dye for the test.

Test for protein is made by adding soluble protein to 3 ml of the reagent.

VIS spectrometry is then used to detect the wavelength shift relative to 3rd of the reference reagent.

Adequate time for stabilization of both the reagent and the protein sample change must be allowed for (~ 10 min).

There are actually three ways that VIS spectroscopy reveals protein existence

1. Right shift of the reagent frequency
2. Red dye peak @ ~ 525 nm is removed from the spectrum because of reactance w/ the protein
3. The gradient of the spectrum is smoother in the 400 - 440 nm region after reactance w/ the protein w/ a reduction of the inflection point @ ~ 440 nm.

Many ways of showing the protein existence are now available w/ the VIS method & the reagent

Ran two trials:

		50ul dilute	Control
Trial 1	Control	Protein	Red Dye Peak
Peak @	662	662 no shift	524 (very slight)

Only method 2 is discernible here - Unsatisfactory trials

This trial #1 was indeed a failure. Unknown as to why. The color shift is also detectable by eye w/ VIS by quickly and the difference was not discernible by eye either in this case.

Definitely intriguing.

I see two issues. I think that the red dye addition should be doubled. I think our protein solution is now so weak as to not be detectable.

With these 2 changes, Trial 2 proceeds.

Trial #2 Difference is clearly discernible by eye. Control: more green Protein: More purple

Control: Peak @ 661 nm
Red Dye peak @ ~525
small but definite

Protein: Stronger, higher peak @ 645 nm
Red dye peak still evident @ ~527 nm.

By eye we had a shift towards purple.
By VIS we have a shift towards purple.

There was a definite shift

The changes are picture of what to expect.
Dismiss the ~525 peak and the gradient
difference @ ~400-440nm.

These did not hold up.

The most important changes here were the increase
in red dye (100 ul to 200 ul) and an increase
of to protein concentration. These will be held.

The reagent now is:

This is the current protein detection reagent.

- 6 ml H_2O
- 3 2 drops Conc NaOH or KOH
- 2 drops $CuSO_4$
- Tartaric acid sufficient to clarify.
- 200 ul of dilute red dye #3 (1 drop in 30 ml H_2O)

+ sufficient protein (soluble) concentration to be detected.

Run again. Remember to wait sufficiently for color development
w/ the protein added. The will need to be
standardized (5-10 min)

Trial #3 (Slightly discernible by eye w/ 3 min)
Control Peak @ 662 nm OK.
Slight red dye peak @ ~525 nm OK.
Protein Peak @ 655 nm definitely discernible but smaller
Red dye peak @ 526 nm. shift.

We do have a ~~the~~ viable defined method of protein detection now, that is repeatable under controlled reagent conditions.

I would increase KOH / NaOH to 3 drops however.

Now we go on to a difficult test (w.r.t. solubility of the COB protein).

[Incidentally, a third test w/ a peak shift of ~5 nm was obtained ~~3~~ also slightly discernible by eye. It is a sensitive test but delicate to conduct w/ strict controls required.

A zoom in run between 650 - 670 nm to be called for here.]

OK, we have some really good news here, in a double decked form.

First, our digested culture based upon the HEPES filter culture, which we presumed as highly proteinaceous (which will soon be verified)

IS HIGHLY SOLUBLE IN MILDLY ACIDIC SOLUTION

This is highly valuable

Increase range from 500 nm to 700 ~~680~~ nm now.

The same material (ie highly proteinaceous) is INSOLUBLE in alkaline solution.

This is an extremely important property of the particular protein.

Next we now conduct our developed protein detection test on the slightly acidified (presumed) protein. (50 ul added).

Result: By eye, we have a major color shift

* TO A BOLD PURPLE. This is indeed highly concentrated proteinaceous material.

Next we will run the VIS spectroscopy for a record of the now established fact.

Culture Protein Detection Trial:

Control Reagent: Peak @ 662 nm OK ✓

CDB Culture Candidate: Peak @ 554 nm

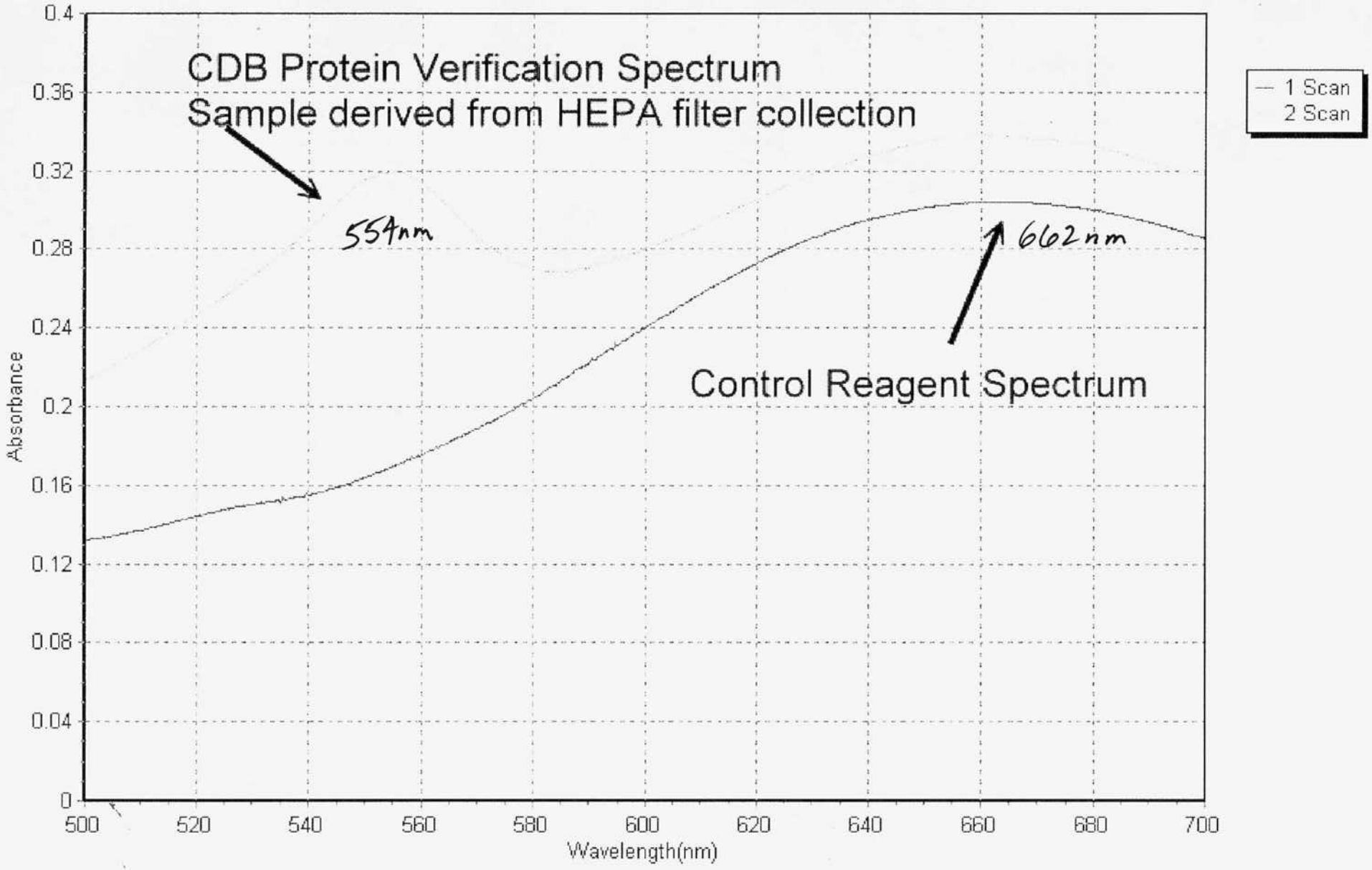
A major shift in absorbance here.

Conclusion: The CDB (microwave digested) culture material (based upon a HEPA seeded culture)

IS HIGHLY PROTEINACEOUS.

COB Protein Verification
Based upon HEPA filter collection - culture

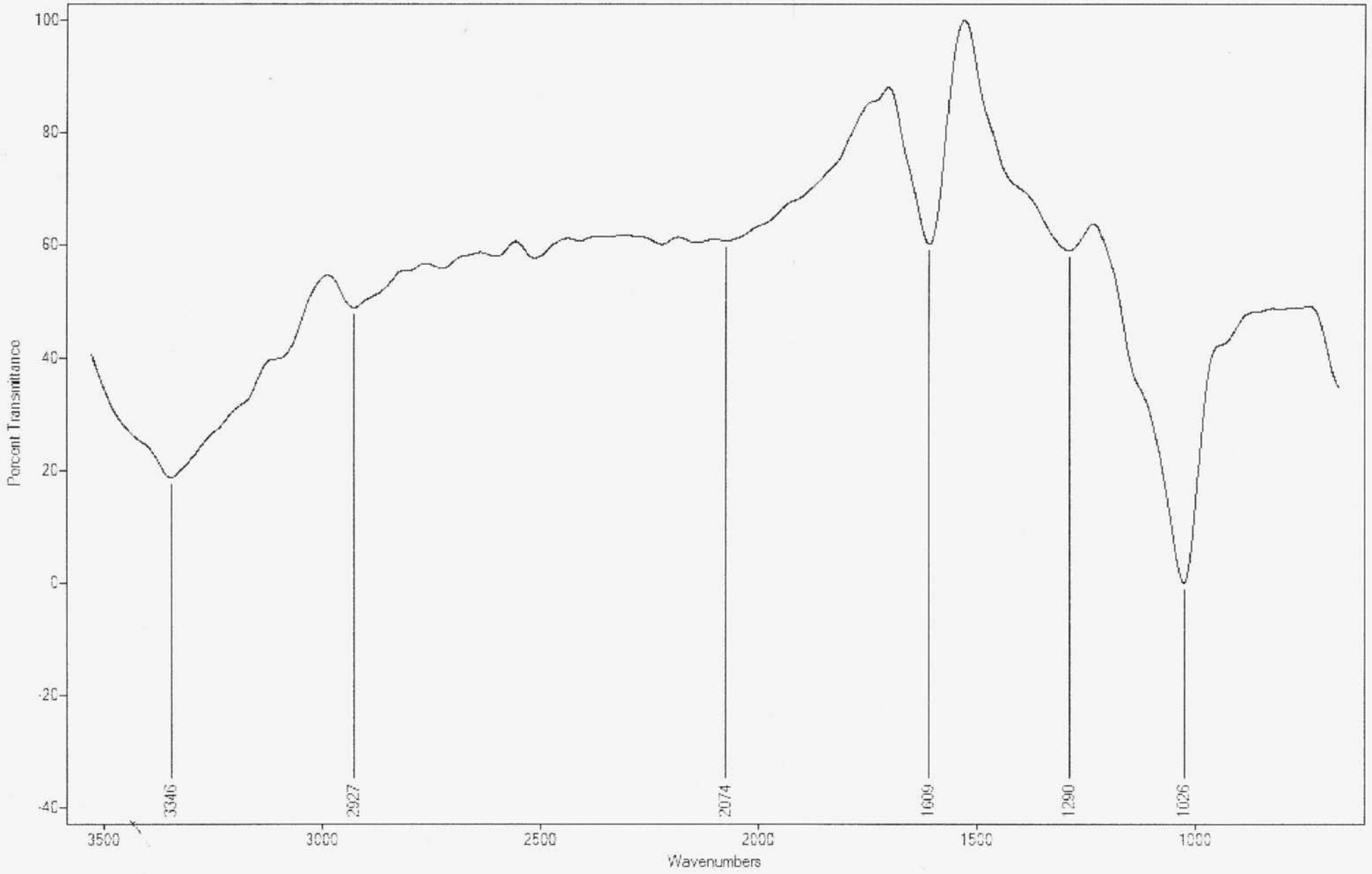
CDB Protein Test Nov 21 2018 - 01.jpg



Same sample - IR analysis
Closest match is the Secreted - Viscous protein CDB
r=.88

Page 143

CDB HEPA Culture Digested - Neutralized Nov 21 2018 - 02.spc: Synthetic spectrum by averaging. See audit trail for details.



printed on 11/22/2018 00:28:49

Nov 22 2018 Thanksgiving Day

Many many topics coming up.

✓ 1. What is the pH of the culture?
2. What is the ORP of the culture
✓ 3. Consider a source by phosphorus for the culture.
Remember SPONCH?
Sulfur Phosphorus Oxygen Nitrogen Carbon Hydrogen

✓ 4 Simple propagation index can be developed
5. The sputum discovery is worth a paper

✓ 6. The developed protein test is quite remarkable

✓ 7. The culture that has been subjected to a loop sweep of 100 – 900 MHz electromagnetic energy is showing signs of disruption

This is potentially quite important.

✓ 8. The culture subjected to ultrasound shows no obvious disruption.

✓ 9. Monitor the cultures in general.

10. The blood slide is worthy of examination.

11. DNA project based upon recent culture work

12. The skin examination project

13. Molecular weight project
14. Toxicology project

Page 145

Propagation Index: ~2018-2019 $\left[\left(\frac{SFI}{Ap+1}\right) \cdot \underset{(1,2,3)}{\text{Imogram Density}}\right] \cdot 1.5$ Current $\left[\left(\frac{69}{5}\right)(2)\right]1.5 = 41$

Approx Range = 0 to 100

Let's start simple w/ the radio propagation index:

Propose: $\left(\left[\frac{SFI}{(Ap\ Index)+1}\right] \cdot \text{Imogram Density}\right) * \text{Desired Scaling Factor}$

Imogram Density = 1 (Low), 2 (Medium), 3 (High)

In the absence of Imogram data, assume a density of 1

Expected range for the immediate future, ie during the next year

SFI hard to imagine exceeding 100, currently @ 69
Ap Index has reached a max of ~30 this year

We are therefore dealing with a max expected of

$\sim\left[\left(\frac{100}{0+1}\right) * 3\right] \cdot X = 100 \qquad X = 1/3$

Almost impossible to reach the max under current & projected conditions.

A typical value now would be $\left(\left(\frac{69}{5+1}\right) * 2\right)\left(\frac{1}{3}\right) = \underline{7.7}$

A more reasonable current scale factor would therefore be $\sim 5\left(\frac{1}{3}\right) = \frac{5}{3}$

Very poor Conditions would be $\left(\left(\frac{69}{25+1}\right) * 1\right)\left(\frac{1}{3}\right) = 0.9$

Let's use 1.5

Favorable: $\left(\left(\frac{69}{2+1}\right) * 3\right)\left(\frac{1}{3}\right) = 22$

Current: $\left[\left(\frac{69}{5}\right)2\right]1.5 = \underline{41}$

Next: Source of phosphate

Ammonium Phosphate seems to be a readily available fertilizer.

I do not have ammonium phosphate

but I do have Sodium Phosphate Monobasic
NaH_2PO_4

Does have toxicity issues
Acts as a laxative
Has a pH of 4.5, melting point 212° F (Ouch)

Dibasic has a pH of 9.5

Let's monitor the current culture, then consider a next generation w/ NaH_2PO_4 added.

Calibrate pH meters:

Not True

We learn that the pH meters are no longer responsive. New meters required.
You will have to use pH paper for now.

With paper, the pH of the HEPA culture is essentially neutral.

The pH meters are both fine.

Calibrate them with

	pH
Bleach (Household)	~13
Ammonia (Household)	~~13~~ 11.5
Vinegar	3

Actually the meters are fine in the presence of a significant acid or base, but they are not very responsive from pH ~ 5 to 9 so they are actually poor for more exact work.

The meter appear to be satisfactory. Neutral pH's are ~ from 6 to 8.

OK, on we go.

Let's look @ the seeded HEPA culture status. This is approx. 3rd generation now.

The seeded culture is fully developed w/ a major filamat network combined w/ protein conglomerates. Microphotographs taken.

I will harvest this culture and begin two new seeded cultures, one will contain sodium phosphate this time, however.

The 3rd generation seeded HEPA cultures are most certainly quite pure. The two large cultures (10" x 10") were both highly matted and this means that a highly developed filament network is in place. This is a higher tier of growth than the restricted COCCUS form that was developed in broth prior.

The rate and volume of production is high w/ the current methods; they should be amenable to the DNA extraction trials that are in planning.

It is also apparent that the frequency sweep is having a degrading influence upon the culture. The current level may be high however as the pencil electrodes are being obliterated in the process.

I will see if I can drop the voltage, however this will force me to adopt a single voltage applied frequency without a sweep function!

Another observation is that the culture medium becomes clarified, another indication of growth inhibition!

We may need to consider nail (iron) electrodes however, as they will be more congruent to the existing culture vs. the introduction of a large volume of graphite.

Let's look @ the culture results under the scope - approx 2 days have passed here.

There clearly is a degradation in the culture growth. The filament network is not developing - the frequency sweep (100-900 MHz) (Δ = 100 MHz, 10 mins per freq) clearly is preventing the filament growth from taking place.

There still remains conglomeration of the CDB, and potential protein conglomeration, however it definitely appears to be curtailed. The density of the culture overall is decreased. There also is no development of the "cellular" units that are observed within the more recent advanced cultures.

This suggests that there is good cause established for further electromagnetic research and its effect upon culture growth.

I will switch to iron electrodes on the next trial.

A report on the culture subjected to ultrasound energy @ 5 MHz.

Greater protein / COB conglomeration is taking place in the culture that has been subjected to the frequency sweep.

In addition, the "cellular" units are appearing in the ultrasound culture, where none were visible in the frequency sweep culture.

The ultrasound culture also has greater density and appears more visual with a cloudy medium vs the frequency sweep culture.

At this point there is no strong reason to continue w/ the ultrasound experiment on the culture.

A second trial of the freq sweep from 100 to 1000 MHz is being conducted w/ new electrode. with a newly established culture

For thoroughness sake, I have also established a "Control Culture". In this case, the medium was created but no explicit addition of a active CDB-filament "seed" was added to the medium.

We see:

1. Possible evidence of CDB conglomeration
2. Likely evidence of iron oxide film production
3. No filament evidence
4. No bacteria culture development apparent

The interpretation of this as follows. Evidence shows that the CDB are literally omnipresent. They also appear to sustain themselves indefinitely in any environment. Growth of the CDB-filament network appear to depend primarily upon the suitabilities of the medium. More suitable mediums will allow for more advanced growth forms as well as a rate of growth increase.

We must recall that even in the original patent application note was made of filament growth in "water" over prolonged periods of time. This has been observed in various flask setups (pumps, water, distillation, etc) over prolonged periods of time (eg months).

I have no ability to create a completely "sterile" environment here and I suspect it will be quite difficult to achieve most anywhere. What is effected, by the medium is the rate, the volume and the overall complexity of the subsequent growth.

I now have two electromagnetic (EM) cultures set up. Steel electrodes (nails) are being used.

The first is a sweep (100-1000 MHz)

The second is a single frequency @ 500 kHz (.5 MHz)

With a meter, we learn that the sweep culture is pushing about a 50 uA current through the medium. This is doing a heck of a number on the electrodes, and it makes it more difficult to assess. I can not adjust the current or voltage, it is fixed.

The second culture, single frequency, is pushing about ½ uA of current through the cell. This is causing no major reaction of any kind with the electrodes of that set.

Only the sweep culture presented any observable change in the culture, so that's so far, since it is the only trial that has been made.

Nov 23 2016

We have something most unusual and unexpected taking place. It is with respect to (w.r.t.) inhibition of culture growth taking place.

I have added phosphates to a seeded HEPA culture to observe any effect. The culture is now 3 days old — very recently developed to show the "cellular" units forming along with the beginnings of the filament network. The control culture is developing perfectly normally.

In contrast, the culture w/ sodium phosphate monobasic (NaH_2PO_4) added has been severely inhibited in growth. This was not expected in any fashion, in fact, the converse was expected.

In addition, the culture turned white rather quickly negating the normal rust color appearance from iron oxidation.

Photographed @ 800x

The "cellular" units and the filament network IS NOT @ all developing in the culture with the phosphate compound added. I suspect I added about ½ to ½ teaspoon to the 10" x 10" culture. I will now decrease to a marginal level and repeat the culture process.

We may have an additional important inhibitor to culture growth, as we found w/ citrate, Vit C, & NAC

Let's look up on the toxicity of NaH_2PO_4

It acts as a laxative
It acts as a buffer
pH is 4.5

Sodium phosphate buffers exist in all cellular fluids

Monobasic, in combination with dibasic, acts as a laxative.

Can be harmful if swallowed or inhaled.
Can cause electrolyte imbalances
Severe symptoms can result from sodium phosphate enemas

Effects cattle - increases sperm count & volume from 8-10 grams added per day. 45 day results

Colonoscopy preparation.

At one time 250-1000 gm doses were given to horses to acidify urine.

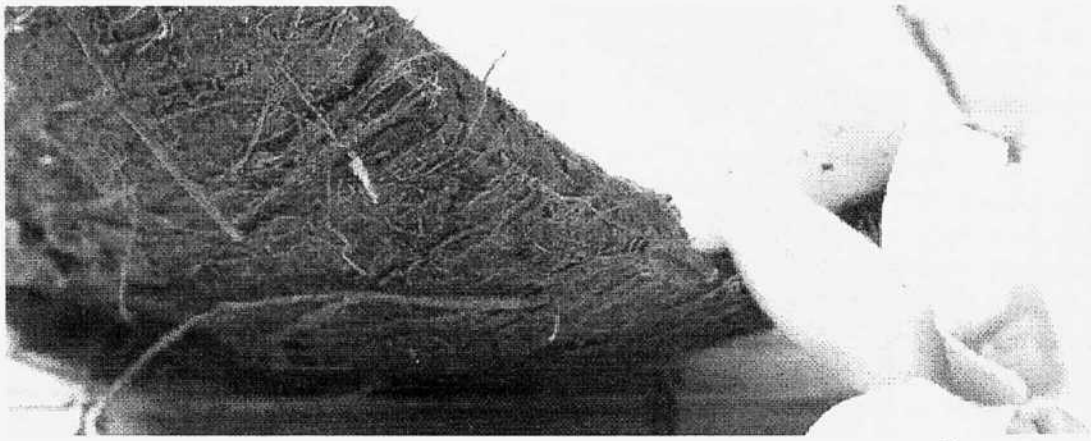
Kidney injuries from laxatives are on record.
"Oral sodium phosphate products" OSP's.

Kidney issues are important in any administration.
Electrolyte abnormalities in the elderly.

Phosphorus / Phosphates

Page 156

Page 156 A



Phosphorus, Phosphate

Also listed as: Phosphates, Fleet Phospho-soda®, Fleet Enema

Related Terms

- Aluminum phosphate, anhydrous sodium phosphate, bone ash, bone phosphate, calcium orthophosphate, calcium phosphate, calcium phosphate dibasic anhydrous, calcium phosphate dibasic dihydrate, calcium phosphate tribasic, colestilan, dibasic potassium phosphate, dibasic sodium phosphate, dicalcium phosphate, dicalcium phosphate, dipotassium hydrogen orthophosphate, dipotassium monophosphate, dipotassium phosphate, disodium hydrogen orthophosphate, disodium hydrogen orthophosphate dodecahydrate, disodium hydrogen phosphate, disodium phosphate, elemental phosphorus, MCI-196, monobasic potassium phosphate, monobasic sodium phosphate, neutral calcium phosphate, phosphate of soda, phosphatidylcholine, phosphatidylserine, potassium acid phosphate, potassium biphosphate, potassium dihydrogen orthophosphate, potassium phosphate, precipitated calcium phosphate, sevelamer (Renagel®), sodium orthophosphate, sodium phosphate, tertiary calcium phosphate, tribasic sodium phosphate, tricalcium phosphate, whitlockite.
- **Note**: The term "phosphates" in this monograph refers to anhydrous sodium acid phosphate, dibasic sodium phosphate, dipotassium phosphate anhydrous, monobasic potassium acid phosphate, monobasic sodium phosphate, phosphorus, potassium phosphate, sodium biphosphate, and sodium phosphate. Phosphate salts should not be confused with toxic substances such as organophosphates, or with tribasic sodium phosphates and tribasic potassium phosphates, which are strongly alkaline.

Top

Page 156 B

Background

- Phosphorus is a mineral found in many foods, such as milk, cheese, grains, dried beans, peas, colas, nuts, and peanut butter. Phosphate is the most common form of phosphorus. In the body, phosphate is the most abundant intracellular anion. It is critical for energy storage and metabolism, the utilization of many B-complex vitamins, the buffering of body fluids, kidney excretion of hydrogen ions, proper muscle and nerve function, and maintaining calcium balance. Phosphorus is vital to the formation of bones and teeth, and healthy bones and soft tissues require calcium and phosphorus to grow and develop throughout life. Inadequate intake of dietary phosphate can lead to hypophosphatemia (low levels of phosphate in the blood), which can lead to long-term potentially serious complications. Conversely, excess phosphate intake can lead to hyperphosphatemia (high blood phosphorus levels), which occurs particularly in people with impaired kidney function and can lead to potentially serious electrolyte imbalances, adverse effects, or death.
- In adults, phosphorus makes up approximately 1% of total body weight. It is present in every cell of the body, although 85% of the body's phosphorus is found in the bones and teeth.
- Phosphates are used clinically to treat hypophosphatemia and hypercalcemia (high blood calcium levels), as saline laxatives, and in the management of calcium-based kidney stones. They may also be of some benefit to patients with vitamin D-resistant rickets, multiple sclerosis, and diabetic ketoacidosis (a very serious complication in which the body only uses fatty acids as fuel and produces acidic ketone bodies).
- Based on the potential for side effects associated with high blood levels of phosphorus, phosphorus supplementation should be done only under medical supervision.

Top

Evidence Table

These uses have been tested in humans or animals. Safety and effectiveness have not always been proven. Some of these conditions are potentially serious, and should be evaluated by a qualified healthcare provider.	GRADE *
Occasional constipation is a use of phosphates approved by the U.S. Food and Drug Administration (FDA) in adults and children, both in oral form and as an enema (for example, Fleet Enema). Phosphates are also used to restore bowel activity after surgery.	A
Phosphate salts (except for calcium phosphate) are effective in the treatment of hypercalcemia. However, intravenous phosphate for treating hypercalcemia may not be recommended, due to concerns about lowering blood pressure, excessively lowering calcium levels, heart attack, tetany, or kidney failure. Sudden hypotension (low blood pressure), kidney failure, and death have been reported after phosphate infusion.	A
Hypophosphatemia is an FDA-labeled use of phosphates in adults. Taking sodium phosphate or potassium phosphate is effective for preventing and treating most causes of hypophosphatemia and should be directed under medical supervision. The underlying cause of the hypophosphatemia should be identified and corrected whenever possible.	A
Kidney stones (nephrolithiasis) are an FDA-labeled use of phosphates in adults. Taking potassium and sodium phosphate salts orally may help prevent kidney stones in patients with hypercalciuria (high urine calcium levels) and in patients with kidney stones made of calcium oxalate. However, phosphate administration when stones are composed of magnesium-ammonium-phosphate or calcium phosphate may increase the rate of stone formation.	A
This is an FDA-labeled use of phosphates in adults and children. Sodium phosphate taken orally or as an enema may be used for bowel cleansing in preparation for surgery, imaging studies, or endoscopy (for example, Fleet Phospho-soda®, Fleet Enema). Phosphates appear to increase peristalsis and cause an influx of fluids into the intestine via osmotic action. Aluminum phosphate is used orally to neutralize gastric acid.	A
After periods of severe malnutrition or starvation (for example, anorexia nervosa), intravenous phosphate may be necessary in order to prevent a refeeding syndrome. Phosphate levels should be closely monitored in such patients.	B
Early research shows that high amounts of phosphorus may have negative effects on bone density. This is because phosphorus decreases bone formation and increases bone resorption. In clinical research, there was a lack of an association between milk intake and hip fracture in women. Milk is a source of	C

156 C

phosphorus, as are calcium, protein, and supplementary vitamin D in certain countries, such as the United States. Well-designed studies are needed to confirm these findings.

Patients with serious burns may lose phosphate, and replacement may be necessary. Well-designed clinical trials are necessary before conclusions may be drawn.	C
The use of prophylactic phosphate therapy in diabetic ketoacidosis (a very serious complication in which the body only uses fatty acids as fuel and produces acidic ketone bodies) is controversial and may be considered, particularly in cases of low phosphate levels. In general, phosphate replacement is not routinely recommended, based on the lack of clinical benefit in some studies, as well as the potential for adverse effects, such as hypocalcemia and soft tissue calcification. In cases of low phosphate levels, some potassium replacement may be provided as potassium phosphate. Well-designed clinical trials are still necessary.	C
Evidence is mixed with respect to the effect of oral phosphates on exercise performance. Further research is needed.	C
Long-term, slow-release neutral potassium phosphate has been shown to reduce calcium excretion in subjects with absorptive hypercalciuria, and it appears to be well tolerated. This use of phosphates may be considered to prevent kidney stone formation. Further research is required.	C
Hyperparathyroidism is the overactivity of the parathyroid glands. This results in excess production of parathyroid hormone (PTH), involved in the regulation of calcium and phosphate levels. At least in some patients with hyperparathyroidism, serum phosphate levels are low. However, well-designed clinical trials investigating the use of phosphates for this purpose are lacking, and further research is required.	C
The effect of the addition of calcium and phosphorus to human milk on growth and bone metabolism in preterm infants is unclear. Further research is needed.	C
Critically ill patients receiving intravenous feedings often have low phosphate levels. Phosphate levels should be closely monitored in such patients, particularly if kidney function is impaired. Inorganic phosphates avoid incompatibility with calcium in TPN solutions. The addition of phosphate to TPN solutions should be under the supervision of a licensed nutritionist.	C
Vitamin D-resistant rickets is a fairly common type of rickets and is defined by its resistance to treatment with vitamin D. Low levels of phosphates are common in many of these patients. However, well-designed clinical trials investigating the use of phosphates for this purpose are lacking, and further research is required.	C

*** Key to grades**

A: Strong scientific evidence for this use
B: Good scientific evidence for this use
C: Unclear scientific evidence for this use
D: Fair scientific evidence for this use (it may not work)
F: Strong scientific evidence against this use (it likley does not work)

Top

Tradition / Theory

The below uses are based on tradition, scientific theories, or limited research. They often have not been thoroughly tested in humans, and safety and effectiveness have not always been proven. Some of these conditions are potentially serious, and should be evaluated by a qualified healthcare provider. There may be other proposed uses that are not listed below.

- Anxiety, appetite stimulant, bone diseases (pain), cancer, cancer (clear cell carcinoma), dental conditions, depression, encephalopathy (hypophosphatemic encephalopathy), fatigue, growth, irritability, joint problems, multiple sclerosis, muscle pain, osteoporosis, radioactive (thallium) parathyroid scanning enhancement, uterine cancer (uterine papillary serous carcinoma), weight gain, weight loss.

Top

156 D

Dosing

Adults (18 years and older)

- The recommended daily intake has been suggested to be 700 milligrams of phosphorus daily for adults aged 18 years and older, including pregnant or breastfeeding women.
- The tolerable upper intake level (UL) for adults 19-70 years old is four grams daily; for adults more than 70 years old, the UL is three grams daily. The recommended UL in pregnant women is 3.5 grams daily, and in breastfeeding women, it is four grams daily.
- Phosphate salts should not be given to patients with hyperphosphatemia (high blood phosphorus levels) and should be used cautiously in those with impaired kidney function.
- Doses typically range from one to three grams of phosphorus (as a phosphate salt (sodium phosphate or potassium phosphate) or elemental phosphate) daily by mouth for the treatment of calcium oxalate kidney stones, hypercalcemia, or hypophosphatemia. Doses are usually divided and taken throughout the day.
- Fleet Enema (118 milliliters) can be used as a laxative when administered rectally. It should be administered as a single daily dose. Laxatives should not generally be used for more than one week. 4-8 grams of sodium phosphate dissolved in water has also been used as a saline laxative (it should be taken with plenty of water).
- Intravenous phosphate 50 millimoles (sodium: 81 millimoles, potassium: 9.5 millimoles) over 24 hours has been used during refeeding syndrome when serum phosphate falls below 0.5 millimoles per liter. Phosphate blood levels should be closely followed.

Children (younger than 18 years)

- The recommended daily intake for infants and children is: infants 0-6 months old, 100 milligrams (additional phosphorus may be added to infant formulas); infants 7-12 months old, 275 milligrams; children ages 1-3 years old, 460 milligrams; children ages 4-8 years old, 500 milligrams; children ages 9-18 years old (including pregnant or breastfeeding females), 1,250 milligrams.
- The Tolerable Upper Intake Level (UL) for infants aged 0-12 months old is not clearly established and the source of intake should be from food and formula only; for children 1-8 years old the UL is 3 grams daily; for children 9-18 years old the UL is 4 grams daily.
- Children under 12 years of age should not receive an adult-size Fleet Enema. Children 2-12 years of age may receive a Fleet Ready-To-Use Enema for children in a single daily dose (two fluid ounces). Laxatives should not generally be used for more than one week.
- Children 5-10 years old may receive five milliliters of Fleet Phospho-soda® and should not exceed 10 milliliters in a 24-hour period. Children 10-12 years old may receive 10 milliliters and should not exceed 20 milliliters in a 24-hour period. Children over 12 years old may receive a dose of 20 milliliters and should not exceed 45 milliliters in a 24-hour period. Do not administer Fleet Phospho-soda® to children under five years of age.
- Children may also receive intravenous preparations, which should be given under the supervision of a licensed healthcare professional.

Top

Safety

The U.S. Food and Drug Administration does not strictly regulate herbs and supplements. There is no guarantee of strength, purity or safety of products, and effects may vary. You should always read product labels. If you have a medical condition, or are taking other drugs, herbs, or supplements, you should speak with a qualified healthcare provider before starting a new therapy. Consult a healthcare provider immediately if you experience side effects.

Allergies

- Avoid if allergic to any ingredients in phosphorus or phosphate preparations.

Side Effects and Warnings

- In general, sodium, potassium, aluminum, and calcium phosphates are likely safe when used orally in recommended doses for short-term periods by people without hyperphosphatemia, impaired kidney function, or other health conditions known to increase the risk of hyperphosphatemia. Sodium phosphate is likely safe when used rectally for short-term periods in otherwise healthy individuals with normal kidney function. Long-term use or high doses used orally or rectally require monitoring of serum electrolytes. Intravenous phosphate is likely safe when used as an FDA-approved prescription drug under medical supervision in people without hyperphosphatemia, impaired kidney function, or other health conditions known to increase the risk of hyperphosphatemia.
- Nausea or gastrointestinal irritation can occur. A reduction in dosage may be necessary to minimize diarrhea. Potassium acid phosphate may cause dyspepsia in patients with a history of peptic ulcer disease. Aluminum phosphate may cause constipation. Oral sodium phosphate may cause bloating, cramps, abdominal pain, and nausea.
- Phosphate salts should not be confused with toxic substances such as organophosphates, or with tribasic sodium phosphates and tribasic potassium phosphates, which are strongly alkaline.
- Use cautiously in patients with gastrointestinal disorders, burns, pancreatitis, underactive parathyroid glands (with sodium phosphate or potassium phosphate), underactive adrenal glands, or liver disease, as excessive intake of phosphorus or phosphate may worsen these conditions.
- Use cautiously in kidney stone formers.
- Use phosphate enemas cautiously, following medical and label directions.
- Use cautiously in patients with low blood pressure, or in those taking blood pressure-lowering agents.

156 E

- Use cautiously when using agents that may affect electrolyte levels.
- Use cautiously in patients at risk for osteoporosis, rickets, or osteomalacia (softening of bones), as early research shows that high amounts of phosphorus may have negative effects on bone density. Excessive phosphorus or phosphate supplementation may worsen these conditions.
- Avoid in patients with kidney disease, and in those on dialysis, at risk for cardiovascular disease, or using prescribed phosphate binders, due to the increased risk of cardiovascular disease associated with increased phosphate levels, as well as due to the increased risk of parathyroidectomy. Excessive intake of phosphates may cause calcification of kidney tissue or acute kidney failure.
- Avoid excessive amounts, and avoid use in patients with electrolyte imbalances, as excessive intake of phosphates may cause potentially serious or life-threatening toxicity or electrolyte disturbances, such as hypocalcemia (low calcium blood levels), hypomagnesemia (low magnesium blood levels), hyperphosphatemia (high phosphorus blood levels), or hypokalemia (low potassium levels). Death has been reported in infants or adults with oral, rectal, or intravenous phosphates, particularly in those at increased risk for electrolyte disturbances. Late symptoms may include abdominal pain, vomiting of phosphorescent materials, bloody vomiting and diarrhea, headache, limb aches, tongue coating, foul breath, weakness, and yellow conjunctivae (whites of the eyes). Rare complications may include confusion, convulsions (seizures), headache, dizziness, numbness, tingling, pain, weakness, anxiety, increased thirst, muscle cramps, or fatigue. Abnormal heart rhythms, shortness of breath, foot or leg swelling, and weight gain have been reported.
- Avoid with known allergy to any ingredients in phosphorus or phosphate preparations.
- Avoid in pregnant women, especially those with toxemia of pregnancy, or lactating women, unless under the guidance of a health professional.

Pregnancy and Breastfeeding

- The U.S. Food and Drug Administration (FDA) has categorized phosphorus as Pregnancy Category C. The tolerable upper intake level (UL) for phosphorus in pregnant women is 3.5 grams daily, and in breastfeeding women, it is four grams daily. The recommended daily intake in pregnant or breastfeeding females 18 years old and younger is 1,250 milligrams daily.

Top

Interactions

Interactions with Drugs

- Antacids containing aluminum, calcium, or magnesium can bind phosphate in the gut and prevent its absorption, potentially leading to hypophosphatemia (low phosphate levels) when used chronically.
- Some anticonvulsants (including phenobarbital and carbamazepine) may lower phosphorus levels and increase levels of alkaline phosphatase.
- Bile acid sequestrants such as cholestyramine (Questran®) and colestipol (Colestid®) can decrease oral absorption of phosphate. Therefore, oral phosphate supplements should be administered at least one hour before or four hours after these agents.
- Corticosteroids may increase urinary phosphorus levels.
- Potassium supplements or potassium-sparing diuretics taken together with a phosphate may result in high blood levels of potassium (hyperkalemia).
- Alcohol (ethanol) may increase urinary phosphorus. Wine may enhance absorption of phosphorus (as well as calcium and magnesium).
- Calcimimetics and insulin may decrease blood levels of phosphorus.
- Estrogen may increase urinary phosphorus.
- Phosphate binders decrease blood levels of phosphorus.
- Medications that may affect electrolyte levels should be used cautiously with phosphates. Examples include amiloride (Midamor®); angiotensin-converting enzyme (ACE) inhibitors such as benazepril (Lotensin®), captopril (Capoten®), enalapril (Vasotec®), fosinopril (Monopril®), lisinopril (Zestril®, Prinivil®), quinapril (Accupril®), or ramipril (Altace®); cyclosporine; cardiac glycosides (Digoxin®); heparins; anti-inflammatory drugs; potassium-containing agents; salt substitutes; spironolactone (Aldactone®); and triamterene (Dyrenium®).
- Phosphates may cause low blood pressure. Caution is advised in patients taking agents that lower blood pressure.
- Phosphates may also interact with ACE inhibitors, cardiovascular agents, gastrointestinal agents, hepatotoxic agents, osteoporosis drugs, and renal agents.

Interactions with Herbs and Dietary Supplements

- Calcium may impair phosphates in the body and result in calcium deposits in tissues.
- Pumpkin seed may increase urine phosphates.
- Niacin might decrease blood levels of phosphorus.
- Excessive doses of calcitriol, the active form of vitamin D (or its analogs), may result in hyperphosphatemia (high phosphate levels).
- Phosphates may cause low blood pressure. Caution is advised in patients taking agents that lower blood pressure.
- Phosphates may also interact with ACE inhibitors, antacids, anticonvulsants, anti-inflammatory agents, bile acid sequestrants, calcimimetics, cardiovascular agents, diuretics, electrolyte-modifying agents, fructose, gastrointestinal agents, hepatotoxins, high-phosphate beverages (such as cola drinks), hormonal agents, magnesium, osteoporosis agents, phosphate binders, potassium, renal agents, and salt substitutes.

Top

156 F

Attribution

- This information is based on a systematic review of scientific literature edited and peer-reviewed by contributors to the Natural Standard Research Collaboration (www.naturalstandard.com).

^ Top

Bibliography

1. Bredle DL, Stager JM, Brechue WF, et al. Phosphate supplementation, cardiovascular function, and exercise performance in humans. J Appl Physiol 1988;65(4):1821-1826.
2. Bugg NC, Jones JA. Hypophosphataemia. Pathophysiology, effects and management on the intensive care unit. Anaesthesia 1998;53(9):895-902.
3. Ehrenpreis ED, Nogueras JJ, Botoman VA, et al. Serum electrolyte abnormalities secondary to Fleet's Phospho-Soda colonoscopy prep. A review of three cases. Surg Endosc 1996;10(10):1022-1024.
4. Fakiris AJ, Moore DH, Reddy SR, et al. Intraperitoneal radioactive phosphorus (32P) and vaginal brachytherapy as adjuvant treatment for uterine papillary serous carcinoma and clear cell carcinoma: a phase II Hoosier Oncology Group (HOG 97-01) study. Gynecol Oncol 2005;96(3):818-823.
5. Fine A, Patterson J. Severe hyperphosphatemia following phosphate administration for bowel preparation in patients with renal failure: two cases and a review of the literature. Am J Kidney Dis. 1997;29(1):103-105.
6. Finn WF, Joy MS. A long-term, open-label extension study on the safety of treatment with lanthanum carbonate, a new phosphate binder, in patients receiving hemodialysis. Curr Med Res Opin. 2005;21(5):657-664.
7. Fisher JN, Kitabchi AE. A randomized study of phosphate therapy in the treatment of diabetic ketoacidosis. J.Clin.Endocrinol.Metab 1983;57(1):177-180.
8. Garg JP, Chasan-Taber S, Blair A, et al. Effects of sevelamer and calcium-based phosphate binders on uric acid concentrations in patients undergoing hemodialysis: a randomized clinical trial. Arthritis Rheum 2005;52(1):290-295.
9. Heaney RP, Nordin BE. Calcium effects on phosphorus absorption: implications for the prevention and co-therapy of osteoporosis. J Am Coll Nutr 2002;21(3):239-244.
10. Helikson MA, Parham WA, Tobias JD. Hypocalcemia and hyperphosphatemia after phosphate enema use in a child. J Pediatr Surg 1997;32(8):1244-1246.
11. Kastenberg D, Chasen R, Choudhary C, et al. Efficacy and safety of sodium phosphate tablets compared with PEG solution in colon cleansing: two identically designed, randomized, controlled, parallel group, multicenter phase III trials. Gastrointest Endosc 2001;54(6):705-713.
12. Kemi VE, Kärkkäinen MU, Lamberg-Allardt CJ. High phosphorus intakes acutely and negatively affect Ca and bone metabolism in a dose-dependent manner in healthy young females. Br J Nutr. 2006 Sep;96(3):545-52.
13. Kuschel CA, Harding JE. Calcium and phosphorus supplementation of human milk for preterm infants. Cochrane Database Syst Rev. 2001;(4):CD003310.
14. Kurihara S, Tsuruta Y, Akizawa T. Effect of MCI-196 (colestilan) as a phosphate binder on hyperphosphataemia in aemodialysis patients: a double-blind, placebo-controlled, short-term trial. Nephrol Dial Transplant 2005;20(2):424-430.
15. National Research Council, Food and Nutrition Board. Recommended Dietary Allowances. 10th ed. Washington, D.C.: National Academy Press; 1989:184-187.

^ Top

^ Top

Density of Monobasic is 2360 kg/cu meter

How many cm^3 in m^3 ? $= (100)^3 = 100{,}000$ (adjustment)

So we have $\frac{2360}{100{,}000}$ gm in one cm^3 $= .024 \frac{kg}{cm^3} = \frac{24 gm}{cm^3}$

seems pretty high, but it seems to be so.

Now what is the volume of a teaspoon? $= 4.93 ml \cong 5 ml$

means $\frac{4.93 ml}{4.80 gms} = \frac{1 ml}{.97 gms}$ Use this

So 1/32 of a tsp = 0.154 ml

We are using 1/4, 1/2, 3/4, Full =
.039 ml, .077 ml, .116 ml, .154 ml

Actual Measurement Based

If we assume 1 ml = 1 cm^3 we therefore have (of a 1/32 tsp.)

1/4	⇒ .039 cm^3 ml = .936	gms .038	I do not
1/2	⇒ .077 cm^3 ml = 1.85	gms .075	believe that
3/4	⇒ .116 cm^3 ml = 2.78	gms .113	we are using nearly
full	⇒ .154 cm^3 ml = 3.70	gms .149	the mark in our

cultures. Weigh it.

Actual Measurement: 1 tablespoon = ~~24~~ 14.4 gms.
Therefore 1 tsp = 1/3 tablespoon = 4.80 gms.

Now we can determine the concentration of the NaH_2PO_4 culture w/ greater certainty.

Each culture is ~100 ml

So we have

1/4 = .038 gms/100 ml = ~~0.38 kg~~ 0.38 gms/kg.
1/2 .075 gms/100 ml = 0.75 gms/kg
3/4 .113 gms/100 ml = 1.13 gms/kg
Full .149 gms/100 ml = 1.49 gms/kg

Now a human body weighs approx.
(1 kg ≅ 2.2 pounds)
(1 pound ≅ 0.45 kg)

Average weight is now 195 lbs (2014) male
It used to be 180 !!!!!!!!!!!!!!
So average weight now is ~88 kg (very high!)

* So our weakest culture equates to approx
33 gms / person

Our strongest culture equate to approx
131 gms / person

We are, fortunately, seeing a dramatic effect upon the culture with even the weakest NaH_2PO_4 solution, but we will want to decrease it even further in the testing.

0.2 to 0.4 gms required for maintenance.

The estimated fatal dose of sodium phosphate is 50 gms.

So obviously, this would need to be dramatically reduced, even in the weakest culture.

The use of crushed bone will be an interesting experiment.

I would surmise that you would need to get down to ~1 gm to become practical.

I see this as being quite flexible since the effect upon the culture is quite dramatic even in the weakest solution used.

Phosphate electrolyte levels would be an important measurement of status therefore. Low phosphate levels would indicate a more likely problem.

Bone problems, fatigue + weakness are symptoms

Cramping

Phosphorus is the 2nd most abundant mineral in the body! after Calcium.

Another way of interpreting the culture results is that the microorganism (CBB) is already and most certainly reacting with phosphorus in the body.

Means that a phosphorus interference is likely or expected to be occurring.

Symptoms:

1. Fatigue (ATP...)
2. Impaired immune function
3. Bone weakness
4. Bone pain
5. Arthritis
6. Nervous system damage
7. Coordination / Balance loss
8. Loss of reflexes
9. Numbness
10. Tremors
11. Tingling
12. Loss of appetite
13. Weight loss
14. Irregular breathing & heartbeat

Many questions here

Chicken or the egg?

I have:

1. Set up a 10"x10" normal HEPA seeded culture (up to about 6th generation by now) to maintain 2 full size cultures active for harvesting purposes.

2. I have set up a more comprehensive phosphate culture trial.

(C) (1) (2) ~100 ml each

(C) (3) (4)

6 cultures

(C) cultures are control, i.e, normally seeded HEPA cultures

(1) adds ~ 1/4 (1/32) tsp NaH_2PO_4

(2) adds ~ 1/2 (1/32) tsp "

(3) adds ~ 3/4 (1/32) tsp "

(4) adds ~ 1 full (1/32) tsp "

These will be observed. Even w/in 10 minutes it can be seen that there is a color shift from rust to white in these cultures.

Nov 29 2018

Monitoring of cultures:

It is clear that all phosphate-CDB-HEPA seeded cultures have a major reaction within, even the weakest of the four. Under the scope (weakest culture is of greatest interest):

On the weakest culture (24 hrs) there is no structure developing. CDB cocci appear visible but no organization or structure taking place. But we must also recall the level approaches a lethal dose of NaH_2PO_4. We will increase by a factor of ~ 30.

Now for control culture (recall only 24 hrs):

The conglomeration of CDB-protein is at a much higher level than the weak phosphate culture so, however, there is no real identifiable organization taking place up in 24 hours.

We need to wait longer. We can, however, only hold the weak culture and replace the stronger culture w/ weaker culture, seeking a reduction on the order of 1/30.
i.e. ~ (1/30) * (1/4) of a (1/32) tsp NaH_2PO_4 to set us closer to ~ 1gm / 80kg

1/4 of 1/32 tsp of NaH_2PO_4 appears to be ~ $\frac{100}{30}$ grains. The means we must get our dosage down to the order of 3-5 grains for the culture. Let's try it.

Before proceeding w/ reduced NaH_2PO_4

Let's observe the powdered lentil culture.

Here the effect appears to be approx midway between that of the control culture and the weakest phosphate culture.

There is still quite promise w/ the use of a natural food source. Also, the lentil culture did change the color of the culture towards white, just like the phosphate cultures do.

Let's try to repeat culture w/ ~35 grains of NaH_2PO_4.

Before proceeding, however, we notice that we now see initial signs of surface filament growth on the oldest 10"x10" culture. The filament growth is of white color on the surface.

This has been observed several times in years past, especially w/ agar cultures as well as the wine cultures, all exposed to air.

The question in those cases was whether or not there were represented a cross contamination of species or a dark colored growth especially would also appear on the wine cultures.

Under these circumstances, it appears to be almost a certainty that the white filament growth on the surface IS NOT cross contamination; but is the same COB-filament growth expressed on the surface when exposed to the air.

Microscopic examination @ 3000x indicates that this is the case. We see, once again, the two primary growth forms - cocci & a filament form (of same dimensions as the liquid culture). It would be worthwhile to try and promote the additional growth form so that it also can be isolated for further study.

We will make agar cultures.

Ok, I have now separated the white surface filament growth and isolated and transferred it to an agar culture based upon the same medium. Incubate @ 85°F.

Now, back to phosphate reduced cultures.

I have created 3 additional phosphate cultures @ reduced dosage

For 80 kg

= 0.91 gms 3 grains / 100 ml $\simeq (3/100)(.38 \text{ gms/kg}) = .011 \frac{\text{gms}}{\text{kg}}$

2.01 gms 6 grains / 100 ml $\simeq (6/100)(\frac{.38}{.75 \text{ gms/kg}}) = .023 \frac{\text{gms}}{\text{kg}}$

3.01 gms 9 grains 100 ml $\simeq (9/100)(.38 \text{ gms/kg}) = .034 \frac{\text{gms}}{\text{kg}}$

and this is where we need to most likely be in terms of human dosages.

We see that, once again, even the 3 grain culture causes an immediate shift in color towards white.

Now we let the cultures set.

Our culture work is now very much under control.

1. HEPA seeded cultures repeatable forever
2. Contamination harvesting from HEPA seeded cultures
3. Raw HEPA cultures under incubation
   - highly filament predominate
4. Drested HEPA filters under incubation
5. Phosphate cultures under observation
6. Surface filament - Agar cultures under observation.

"

Many folks are attracted to "Rife technology"
ie, application of AC E.M.

It can be also, however, the big question is do
you want to be hooked up to a frequency generator
on repeated occasions for who knows how long?

Direct Chemistry / Nanochemistry offers numerous
advantages if inhibition or destruction
can be accomplished that way.

Most especially the integration of any protocol
within a NUTRITIONAL framework such that
it can be incorporated into a lifestyle
as opposed to a specific protocol, at least on
a long term basis.

We have a menu of three methods that show
direct inhibition thus far.

1. Vit C → reduction (oxidation)
2. Citrate Ion
3. NAC → reduction (oxidation)

Amongst the many supplemental complements

A phospholipid examination is now currently
underway.

* EM Sweep (100-1000 MHz) continuous for 3-4 days shows
no repeatable direct inhibition. Same goes
for Ultrasound application @ 5 MHz for 3 days.

Let's consolidate our list of Nov 22 a bit more:

1. Blood slide examination
2. DNA ~~prop~~ prospects?
3. Spleen examination project
4. Molecular weight project
   - osmometer study
5. Toxicology project
6. "Sputum" paper

Extremely good progress recently, but we still have a handful above.

Current Radio Propagation Index Model is

$$\left(\frac{2}{3}\right)\left(\frac{SFI}{Ap+1}\right)\cdot 1.5^{\sqrt{n}} \qquad (n=1 \text{ to } 3 \text{ subjective})$$

Nov 25 2018 Current: SFI = 10 Ap = 3 n = 1.5 Index = ~~24~~ 25 Good

Page 167

The surface filament from the HEPA seeded culture that is now transported to an agar medium is expanding & fluorescing.

Under the scope the CDB interior alignment w/ in some of the tubes can be seen.

We now have a continuation of cultures that lead to the surface growing form.

I have doubled the size of the (2) 10" x 10" HEPA seeded cultures to increase production further.

The phosphate cultures have stopped dead in their tracks with 3, 6 & 9 grains per ~100 ml. There's an amazing sight.

Incidentally, lets alter the Radio Propagation Index to:

$$\left(\frac{2}{3}\right)\left(\frac{SFI}{Ap+1}\right)\cdot 1.5^{\sqrt{n}}$$

S = Solar Flux Index
Ap = Ap Index
n = Imogram Density ($n=1$ to 3) integer not required

Highest possible number for now is $\left(\frac{2}{3}\right)\left(\frac{73}{1}\right)\cdot 1.5^{\sqrt{3}} = $ ~~250~~ 98 very unlikely

Expected no. $\left(\frac{2}{3}\right)\left(\frac{73}{S+1}\right) 1.5^{\sqrt{3}} = $ ~~220~~ 73

$n=1$ Low density
$n=2$ medium density
$n=3$ high density

$(2/3)$ & 1.5 Scale Factors likely fine through 2019

Nov 26 2018

A couple of culture observations today:

1) The HEPA extract culture placed onto an agar medium have created an interesting effect. To starters, each drop of liquid formed on the agar surface is perfectly circular and symmetrical. A little odd & unexpected.

Second, the surface of the drop formation has a very unusual appearance. It truly looks like a plastic coating or cellophane coating has formed on the surface. Very shiny and with wrinkles in the surface.

It is also wise to allow for the fact that the extract is likely to be extremely alkaline, and this may be causing an unusual chemical reaction between the extract and the agar.

Time will tell...

Inhibition Comments:

2) As far as the phosphate treated cultures go, there is positively and absolutely interference to the growth progression. There is no organizational structure in growth taking place.

COB diffuse clusters appear to exist, but no organization, no alignment, no "cellular" formation, no filament formation is taking place.

Even the 3-grain culture shows the full impact ation w/ the change in color of the culture from rust to off-white.

Clearly, the addition of phosphates, presumably @ an acceptable level of dosage for humans, is having a dramatic and inhibitory effect upon the culture growth.

This is highly promising, in addition to previous work that encompasses the addition of

1. Vit C
2. NAC
3. Citrate ion

Nov 27 2018 Page 170

Webinars active today.

First topic is the hydrolysis of various drugs w/ urine testing using enzymes.

The topic of hydrolysis is front and center here.

Definition of hydrolysis is a major takeaway for me here.

Hydrolysis is. - well I look in 3 different books and I get three different definitions

1. A chemical reaction of a compound w/ water.
2. Decomposition or alteration of a chemical substance by water.
3. A reaction in which a compound is split apart in a reaction involving water. Hydrolysis reactions are often catalyzed by acids or alkalis

(in this case of the webinar, by enzymes.

These definitions cover an interesting range.

Hydrolysis and dissolution do differ.
(DISSOLVING)

Dissolving a salt is not hydrolysis, there needs to be decomposition, alteration, splitting etc

Some culture observations:

1. It appears that fresh water for each culture preparation is important to productivity.

2. Surface area of the container for the culture also appears to be important, the larger the better. Adherence of the CDB to a surface appears to be important or helpful to growth production.

3. Stirring the culture after the surface area has been covered also appears to be beneficial to production. It may be that volumetric production can be close to doubled by stirring when the surface area has a complete growth layer.

2nd Weekend on liquid phase extractions
- Let's look up on the micro method

Nov 20 2018

The incubated (heat pad) 10" x 10" HEPA seeded cultures appear to be more productive than non-heated cultures.

Heated cultures are now running @ ~ 25°C. Am hoping to be able to boost it to ~ 27°C.

The filament agar cultures are progressing smoothly.

OK, the topic now is to attempt to determine if a water soluble form of protein is existing within the developed seeded HEPA cultures.

At least three methods of investigation

1. UV absorbance @ 280 nm
2. Colorimetric testing
3. IR analysis - esp for amides

1. UV: We definitely have significant UV absorbance @ 280 nm but it is not a peak absorbance. Peak absorbance in my case are @ ~ 300 nm & 236 nm.

Protein peak absorbance is stated to be @ 280nm & 200nm. 200nm is not accessible to us.

So we may well signal cast protein in the culture medium, but we need to examine them in the other ways as well.

2. Colorimetric

We are engaging the use of our reagent of Nov 20:

6 ml H_2O
3 drops conc NaOH or KOH (10M)
2 drops $CuSO_4$ (0.5M)
Tartaric acid, sufficient to clarify
200 ul of dilute Bradford #3 (1 drop in 30 ml)

3 ml is control, 3 ml is the sample.

Control reagent has a peak @ 667 nm
- this is true.
you also pick up the minor red edge peak @ ~525nm

Now w/ the sample we have an obvious visible color shift towards green. So most definitely a reaction.

Our peak shift is to ≤ 400nm! Very strong, consistent. Our example of Nov 20 2018 from the HEPA filter had a shift to 554 nm.

The chips is therefore even more dramatic than the HEPA filter investigation.

I will screen to find the peak location which is less than 400nm.

There is significant UV absorption going on here as well. I will open up the entire spectrum from 220nm to 1090nm.

Both lamps are on. You did have a UV discontinuity due to failure to turn the UV lamp on.

We see that UV absorbance is very high even w/o the reagent, so this is to be separated off, as it has been.

What we know then so far is:

1. UV absorption indicates likely protein content, however, it is likely mixed in w/ other compounds.

2. VIS spectroscopy definitely seems to indicate significant protein content in the liquid culture medium.

I will also separate off MID IR absorbance here.

Now let's shift the runs to using the reagent as the reference, or blank.

We need to cover these in UV, VIS & Mid NIR

Let's go for UV first: 220-400nm.

These runs should give our most revealing read.
Our discontinuities are too strong here.
Our peak absorbance by the method, however, appears to be @ ~ 350 nm.
This would match w/ our yellow green shift. Why No significant absorbance in the 220-300 region??
Check this again w/ H_2O as the blank.

Remember why the difference!
Your previous UV test was with the culture medium added to water, as it should be.
The test is not valid that you are doing now
Dismiss & restrict the colored reagent ~~test~~ to VIS spectroscopy only. otherwise use H_2O as the reference
The UV test means nothing.

UV & Near IR tests are to be done separately.

Let's go to Near IR - We have what we need to know w.r.t. VIS & UV spectroscopy here.

NIR results -:

We have no identifiable peak NIR absorbance.

What we do have is gradually increasing NIR absorbance from 960nm as we progress towards 700 nm.

Therefore we have

1. UV indication of protein existence
2. VIS indication of protein existence.

extremely strong colorimetric reaction (prove that sugar, iron do not cause this reaction?).

3. No results of myoglobin from NIR

4. Now we go to Super Red IR.

Well, I think we certainly settled that question.

Guess what the closest match is?

The "CDB Secreted Viscous Protein". r=.92

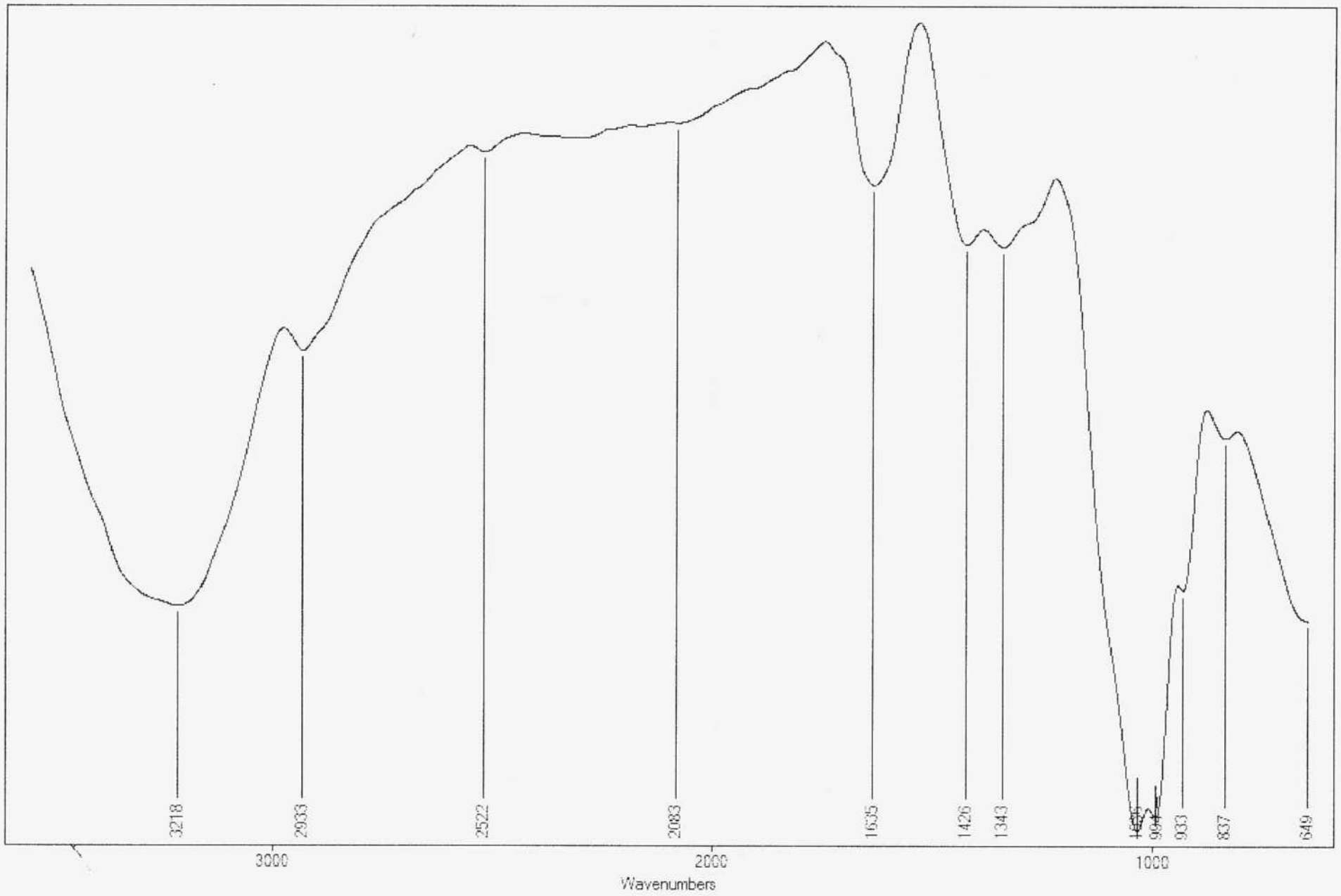
That about says it all.

HEPA Culture - 6th generation - Protein Analysis

Closest Match: "CDB Viscous Secreted Protein" r=.92, .93

CDB HEPA 6th Generation Culture Medium Solution - Protein Investigation Nov 28 2018 - 02.spc: Synthetic spectrum by averaging. See audit trail for details.

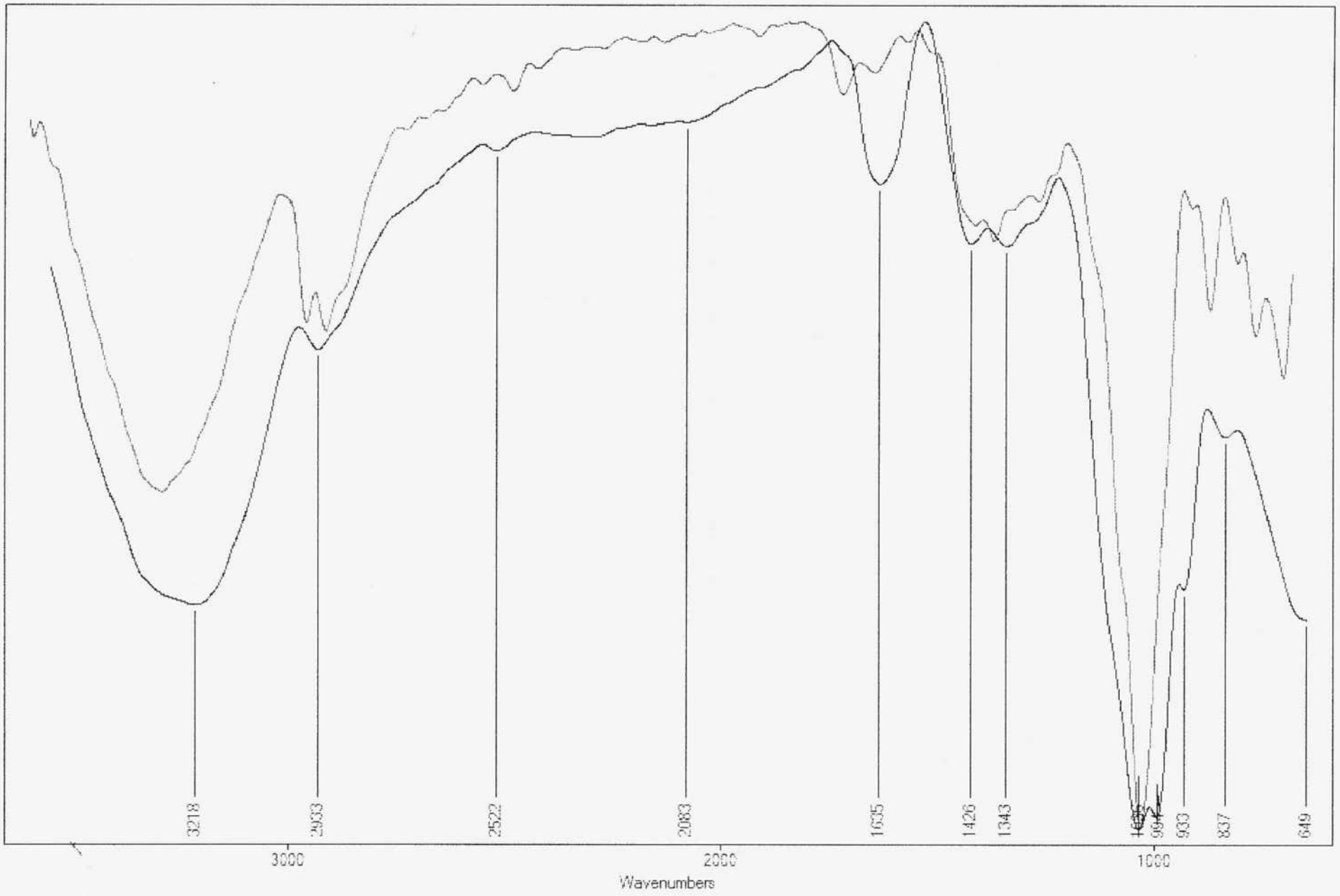
177 A



printed on 11/28/2018 18:05:34

CDB HEPA 6th Generation Culture Medium Solution - Protein Investigation Nov 28 2018 - 02.spc: Synthetic spectrum by averaging. See audit trail for details.

177B



printed on 11/28/2018 18:07:39

A Sea Change is in Place Here Now!

* Well, we defenetely have a protein in solution.

* It happens to be the same protein that was generated by "secretion", or similar process, in prior times.

* What in all ways took weeks to months to accomplish by numerous separate techniques

* Now take place in a matter of days with the improved culture methods in place.

* Within a single process now over the course of 3-4 days we can produce

* 1. The filament structures in solution
* 2 Protein Conglomeration
* 3. Protein organization and structure
* 4. Alignment of COB into filament structure
* 5. "Cellular" protein organization
* 6. Surface filament growth (on 1 week) that can be transferred to an agar medium for continued growth on a solid medium.
* 7. The water soluble protein known as the "secreted" layer, or the red layer.

New finding: save the culture solution after growth, for Condensation & separation

The spectrum also reveals the presence of both the thiocyanate functional group as well as the inorganic cyanide ion (~2520) that is almost certainly due to ferric cyanide,

which also shows up in the IR blood spectrum of an individual (& family) known to be severely affected by the Morgellons condition.

Ferric cyanide, although likely only in trace amounts, can almost certainly be isolated or identified from the current culture medium.

[all very big news here.
One single culture method does it all w/in a few days.]

For kicks now, let's take a sample of the "secreted protein" material (condensed & evaporated) from time past & let's run it through the colorimetric test.

Also, remember the health issues that resulted from pyrolysis of the "secreted protein layer". This is never to be forgotten.

Well, sure enough, the colorimetric test matches perfectly.

We have an immediate and strong color shift of our protein control reagent to a yellow green color.

The exhausted culture medium solution IS THE SAME as the "secreted protein layer" from past incubation cultures that took weeks to develop.

WE HAVE THE SAME PROTEIN IN HAND through a far more efficient & rapid growth technique.

You now may harvest & condense the protein to no end, and start going after its molecular weight via the methods of osmometry & freezing point depression.

—

Strong acid or alkaline (heated by microwave digestion) may dissolve certain portions of the protein structure, but not all! (conglomerate) The polymer network definitely seems to remain. This is at a good problem to settle the details on.

We are now successfully concentrating and purifying the water soluble protein, formerly known as the secreted protein.

We know that the soluble protein is produced directly from the advanced culture process, along w/ the solid form of the protein and the filament network.

I am attempting to determine if the soluble protein produces any volatiles at close to room temperature or under mild heating.

I am getting mixed results. I now have a TVOC (total volatile organic compounds) and I have seen a temporary spike up to ~3 mg/m^3. However, I have gas chromatography (GC) operational again and I am seeing no obvious signals there yet. Ran @ 150°C Hayes-Step D. for 40 minutes.

One drop of the under concentrated protein is now sufficient to produce the colorimetric shift towards yellow green. Coffee filters are also used to purify the concentrate further.

We may have something unusual developing on the agar culture based upon a purified HEPA dissolved extract.

The in the culture where the drops placed look like they are composed of drops of saran wrap that has solidified from a liquid.

It may be that the "wrinkles" or "lines" that were seen w/in the drops after solidification are actually the genesis of filaments.

We may have a case where a filament is emerging from this "film cover".

Well continue to observe.

These cultures are ~4 to 5 days old.

Agar cultures are to be kept fresh w/ a few drops of water added every few days.

We do appear to have volatile compounds within the soluble protein. The TVOC meter registers 5.5 mg/m^3 in a trial, which is quite high and is dangerous to health.

The experimental setup is as follows:

1. Approx 1 liter glass baking dish turned upside down on top of a heating pad.
2. Thermometer inside of meter has thermometer. Temp reached was 35°C.
3. A few drops of the moderately concentrated soluble protein in a watch glass, contained up in the baking glass.
4. The TVOC meter, contained in the baking glass.

The TVOC rose steadily to a max of 5.5 mg/m^3 under these conditions. A control trial will be run w/out the protein included.

A syringe air sample drawn from under the glass rim for transfer into GC. GC comes out generally clean, but there may be a small peak in the pentane region (C_5) of separation. This will need to be verified. $t_r \cong 7.3$ min @ 150°C

N_2, O_2, CO_2 & Argon detected as expected.

Possible C_{10} small spike @ 22.2 min (t_r) @ 150°C

Control underway - heating pad alone may generate volatiles.

We wish to keep the temperature of the heating chamber to ~ 40°C ± maximum. The meter is only safe to ~ 45°C and we have reached 43°C. We will cool down slightly.

The control reaches a max of TVOC of ~2.5 mg/m³ @ 43°C. It should drop a little when we bring temperature of chamber to ~40°C.

Nevertheless, we learn that the heating pad does produce some volatiles and this shows the importance of the control.

Our $\Delta \cong 5.5 mg/m^3 - 2.5 mg/m^3 \cong 3.0 mg/m^3$

This remains a very significant TVOC measurement. Anything above 0.5 mg/m³ is an issue of some kind.

Humidity of control is 14%

—

Now reinsert protein again:

This test will require repetition w/ tight controls.
We show no humidity increase and TVOC of ~2.0 @ temp = 43°C.

Protein removed:

Temp = 43°C RH = 14%

TVOC = 1.3 mg/m³

Δ ≈ 0.1 mg/m³ still significant but we need to reduce variability in results and definitely establish if protein generates TVOC increase @ stable temp & RH.

Nov 29 2018

Webinars today:

1. First is on hydrolysis / detection of opiods in urine - Common methods
   1. Acid Hydrolysis (-Alkaline also)
   2. Enzyme Hydrolysis

Acid method can degrade the compounds
Enzymes will not cause this

Steps will include
1. hydrolysis
2. Extraction
3. Identification

2. Second is detection of opiods in hair "CATCH ES".
3. Third is on the advantage of the QUECHERS System of extraction. Company that produces is UCT
Diabetic reduction in cost, time & increase of accuracy vs SPE method (solid phase extraction)

Very comprehensive extraction technique, often used with foods, environmental
drugs & toxicology are the subjects here.

I have now radically improved the method of collecting & concentrating & purifying the soluble protein form.

What we have here is a case where the culture medium itself is transformed into a protein. This, to me, seems to be a remarkable remarkable event that I would never anticipate w/in any microbiological system. It would literally seem to be unheard of, at least at my level of familiarity.

We now need to:

1. Have a reliable method of determining the sugar consumption rate and extent w/in the culture.
2. Let's run an IR plot on a evaporated sugar solution for an additional point of reference.
3. Perform the colorimetric test w/ sugar also.

The Major List ahead:

1. DNA prospecta
2. Skin examination Project
3. Molecular wt of Protein project
4. Toxicology Project
5. "Sputum" paper — OKV
6. Legacy – Lab notebooks indexed

We have a very very interesting situation that exists with the question of sucrose existence within the soluble protein isolation ~~sss~~ since by all reason we expect some level of sucrose to remain in solution.

We also have confirmed in several ways that the solution does contain a pea protein (soluble) at relatively high concentration, so how do we know how much of each? How do we absolutely prove that we have both in solution?

Two methods are being used.

1. IR analysis
2. Colorimetric reagent test.

Both methods can be used to distinguish between the existence of protein in sucrose, and they both have been used, and they both can be used to establish discrimination.

The colorimetric test creates a strong confirmed color shift in the reagent

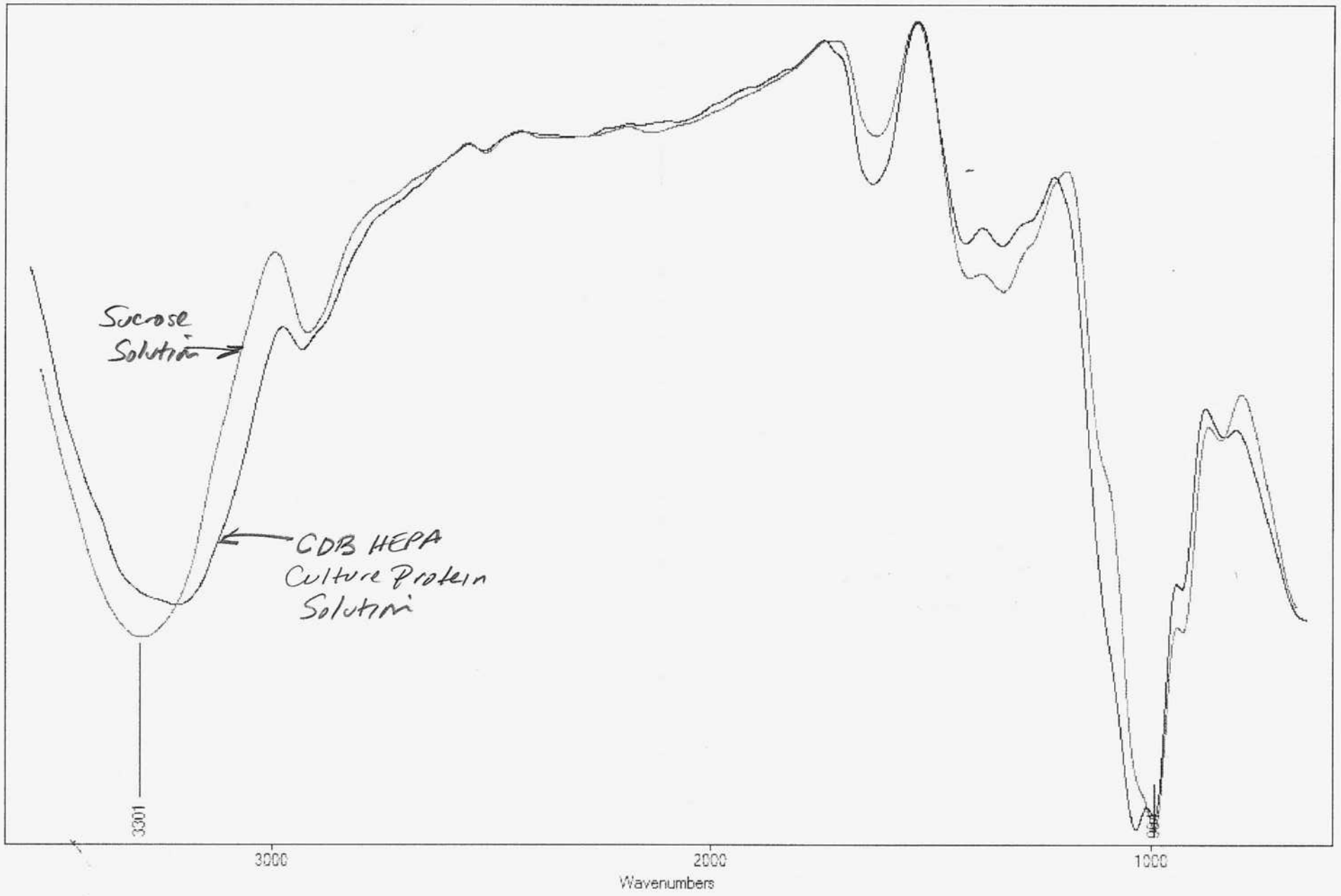
SUGAR does NOT produce the color shift.

IR shows an important distinction in the 3300 Cm^{-1} region.

CDB HEPA Protein Solution (Soluble)
& Sucrose Comparison (ATR)

Sucrose Solution Film ATR Nov 29 2018 - 01.spc: Synthetic spectrum by averaging. See audit trail for details.

189 A



printed on 11/29/2018 14:35:14

The Conclusion is that we are almost certain to have both sucrose and protein in the isolated culture medium.

I will continue to work on the problem of attempting to determine the relative Concentration of each.

Potential methods
1. Polarimetry
2. VIS spectroscopy
   Protein Concentration methods
3. VIS spectroscopy - Color shifts
4. Glucose meters
5. Possible osmometry
6. Index of Refraction (likely the simplest)
7?

For now, we accept the assumption that both sucrose and protein are anticipated to be in the isolated solution.

Start collecting Index of Refraction measurements @ various stages of culture development

3hr culture: Brix 1.6
Filtered Mature Culture (Prior to Concentration) Brix 2.5
Partially Concentrated Filtrate Brix 7.8
Week old Moderately Productive Culture Brix 3.1
IR Random Sucrose Test Solution Brix 11.5

Something to look up on to start with:
The concentration of sucrose vs Brix.

Also in days past we came up with a good model for the protein index of Refraction
(We now understand that this likely includes a sucrose component to it).

One degree Brix = 1 gm sucrose / 100 gms of solution

So a Brix of 1.6 = 1.6 gms sucrose / 100 ml solution (98.4 ml H_2O)

But what you are really measuring is the IOR, not necessarily Brix as they may be (and are) other components beside sugar in the solution.

An advantage of polarimetry is that it may only be responsive to sugar levels, unless the protein itself is chiral.

Recall we have also done w/ with glucose monitoring of cultures in the past.

UV spectrometry may be a practical way to go.

Let's run sucrose by UV.

Ok, we have a great lesson and serendipitous finding here.

Sucrose solution has almost no absorbance, and almost nothing of any significance in the UV spectrum from 220 to 400 nm.

This is obviously very convenient for us.
For solutions

Brix	
0.4	Max absorbance ever
2.1	reached @ 280 nm = 0.11
6.5	usually ≤ 0.08
11.5	

So it looks like absorbance @ 280 nm will be due primarily from the protein.

1 drop of slightly concentrated mature culture medium in a cuvette gives $A_{280} = 0.41$

2 drops: $A_{280} = 0.63$
3 drops $A_{280} = 0.19$
5 drops $A_{280} = 0.90$

So we have obviously determined a method to distinguish sucrose from protein.

Colorimetric tests and UV spectroscopy will easily tell us about the nature of the culture medium.

TVOC Volatility Trial: RH = 14%

Control: Heating Pad T = 40°C TVOC = 1.7 mg/m3

Protein T = 40°C TVOC = 2.6 mg/m3

ΔTVOC = Ø.9 mg/m3 2nd Trial
ΔTVOC Ø.1 mg/m3 1st Trial $\bar{x}$ = Ø.8 mg/m3

The is significant limit it might be difficult to capture the change w/ GC and also to separate it from the control heating pad.

What we tried to elemenate capture of the heating pad.

—

OK, now we place w/in 2 glass pie dishes placed end to end to seal.
The will elemenate most if not all of the heating pad volatiles

Control: Glass Pie Dishes RH: 11% T: 33°C TVOC: Ø.22
Protein RH: 19% T: 34°C TVOC: Ø.64

The appears to be a good measurement of a good control. This leads to ΔTVOC ≅ Ø.4 @ the temperature

Try to obtain a reading w/ a higher temperature.
Notice Humidity Increase

Control: T 40°C RH 11% TVOC .12
Heated Probe: 40 13% TVOC 0.75

Notice PM 2.5 count increased to 80.

So we obviously have volatile in the protein but they require heating to gain access to. We know very well the health risks of Pyrolysis so we must be very careful considering that approach again. This will be impossible w/out a complete fume hood.

So we will need to hold w/ that discovery knowledge for now.

Nov 30 2018

Monitoring certain cultures:
The phosphorus treated culture needs to be examined more closely w.r.t. the control.

The control culture, even with only ~ 100 ml of solution and no inoculation, is a perfect match for the expected growth progression. CDB aggregation & extensive filament network are apparent.

—

O/c the phosphate culture has now been observed after approx one week.

Result: NO NET INHIBITORY EFFECT
UPON THE CULCTURE GROWTH AGAINST
THE CONTROL

For the end, the growth progression is exactly the same - CDB aggregation & extensive filament growth.

It appeared that there was disruption to the culture @ the early stages of growth but after ~ 1 week the original culture and the phosphate treated culture (at least w/ only 3 grains) shows the same growth. The only difference is that the phosphate treated culture turns off white rather than to be rust colored.

You can run the test again w/ increased concentrations, but then you reawaken the toxicity issue.

It would, however, still be of value, from a biochemical interference point of view.

Use 1/32 tsp in ~ 100 ml H_2O & 1/64 NaH_2PO_4.

The tested treated culture (high phosphate content)

also shows no improvement. (i.e., inhibition of growth)

As a matter of fact, the phosphate treated culture visibly shows an even greater matting - filament tendency - it very much has the appearance of a biofilm-filament network. Therefore it is possible that the phosphate has actually enhanced growth, and this was the original supposition and expectation.

I am attempting to see if I can isolate any lipids from the harvested protein - CDB.

I have placed the sample in the blender w/ xylene. Gravity separation afterwards. 4 layers form. There appears to be emulsion like material at a primary brown/tan layer.

I have taken some of the layer and now apply the centrifuge. I once again, appear to have 4 layers.

—

This did not seem to work too well. I do not have any single discernable layer that appears to be of a lipid nature. We likely need to work this over more, possible detergent to be added. It may require a cellular breakdown.
Seems to me we have
1. Xylene (top)
2. Emulsion like material - Not conducive to IR either
3. Water
4. CDB (bottom)

—

Our protein solution is becoming highly concentrated now. Brix (or index of refraction) is now up to 26.
A $\frac{30 \text{ ul}}{3 \text{ ml}}$ solution of ice, a 1% solution is strong enough to produce a full yellow green shift w/ the Colorimedrin test. This is good.

To equate to an earlier solution used in toxicology (0.5%)

We would need $\frac{150\text{ul protein}}{3\text{ml } H_2O} = \frac{X}{100\text{ml } H_2O}$ $X = 5000\text{ul}$

$= 5\text{ml}$

We actually have about 100 ml of protein concentrated now, this means we could make about 2000 ml of 0.5% protein solution for toxicology studies already.

We are doing fine.

We are also headed toward determining the molecular wgt. of the protein.

Dec 01 2018 Page 199

First observation today is that when the Colorimetric protein test sample is allowed to set overnight the color shifts even further to a full yellow from the (original) yellow green.

This means that weak protein solutions may benefit from extended reaction time to produce a color shift (possibly minor).

—

We can see, via the colorimetric protein test, that the phosphate treated culture is definitely producing the protein as well.

A case can be made that the phosphate treated culture is even more productive, as it appears that more solid mass has been created from it. It also appears that a very small amount of phosphate (NaH_2PO_4) is required to produce this result.

I will now separate the cultures into two camps, phosphate treated & non phosphate treated

~1000 ml H_2O	vs	~1000 ml H_2O
1½ - 2 tbs Sucrose (~20 ml)	→	"
½ tbs $FeSO_4$	→	"
½ tsp potato flakes	→	"
½ tsp pink or himalyan salt	→	"
CDB seed	→	"
		⅛ tsp Source of phosphate eg Sodium Phosphate Ammonium Phosphate

(Notes-Sep 01 2020: This is a critical page in the history of CI research, i.e. the introduction of phosphorus into the culture medium.)

Next observation.

The phosphate treated cultures (1/32 & 1/64 tsp) (~100 ml) are extremely productive with the "cellular" units. They are common already on the surface of the liquid culture, and their morphology is only evident under the microscope @ 900x - 3000x

Massive production quickly of the cellular units, which are internally composed of COBs and filament networks.

—

The phosphate cultures now dominate the culture environment underway.

—

"Another Marker for Examination"
(One of Many)

research paper is posted tonight.
The topic is the spectrum examination
- culture comparison work.

The message is fairly straightforward:

They are one and the same.

Dec 02 2018

Since the "Another Mode for Examination" paper is posted, our primary list about revises to:

1. DNA prospects
2. Skin Examination Project
3. Molecular wt of protein project
   - study of Osmometry
4. Toxicology Project
5. Legacy - lab notebooks indexed.

Culture observation:

The HEPA extract culture established on Nov 25 2018 is now starting to show filament growth out from the almost perfectly circular drops. It had taken 1 full week for the process to start. This is an agar based culture.

This culture had the plastic - film like appearance to the drops.

This is a very pure culture, HEPA based, digested and purified w/ filtering to remove all hair & particulates. The drops were highly alkaline from the microwave digestion process.

We now provide two independent methods of initiating a filament culture on agar
1. Liquid culture after exposure to air ~ 1 week
2. Microwave digestion drops ~ 1 week

Time to start studying osmometry theory, freezing point depression theory, and molecular mass determination theory, all based upon colligative properties of solutions.

We need to start w/ a salt solution (NaCl) for initial measurements & calibration

Osmometer Test Solution:

200ml beaker wgt: 111.389gms
111.39 gms

With water added: 215.34 gms Δ=103.96
w/salt added 219.52
Δ= 4.18gms

Therefore we have $\frac{4.18\text{gms}}{103.96+4.18} = .0381 = 3.81\%$ salt solution

Tech: _____
Date: _____ Sam
ple # mOsm
_____
1 1186

Ok we have over 3 first reading.
Now let's make our new 2x & 3x 4 dilutions.

4x solution was too weak.

Not sure why printout says #4 instead of #3 tube.

Try a 3x instead of a 4x.

Number for tube #3 was about 40 hardly linear

I had absolutely no problem whatsoever w/ a 3x solution.

What we have in theoretical is therefore

Tech: _____
Date: _____ Sam
ple # mOsm

1 1234

2 636

New Tubes Prepared

4 Outside Calibration

Rc

MULTI

Tech: _____
Date: _____ Sam
ple # mOsm

1 1237

2 645

Run

% NaCl	mOSm	OSm
3.87%	1235	1.235
1.935%	640	0.640
1.290%	414	0.414

Graph looks fairly linear: yes $r \approx .9993$ Great

$mOSM = 315.742(\%\ NaCl) + 16.269$

$r^2 = .9993$

We appear to be accurate within ≈ 0.01 OSM $= 10$ mOSM.

It looks to be, there's that a 1% salt solution will be within the range of testing for the instrument. The sensitivity will be tested further.

—

Ok, we already have a sense of accuracy for the instrument.

—

Now, the next thing we see is that the instrument actually should be able to read down to 100 mOsm.

This is extraordinarily low.

We can get involved with calibrations. They present 2 pt, 3 pt, 4 pt & 5 pt Calibration standards.

Reference	mOsm
2	100, 500
3	100, 500, 1500
4	100, 500, 1500, 2000
5	100, 500, 1500, 2500, 3000

NaCl Standard Solutions.

We also see how we can develop our own standard solutions if we wish to.

We essentially have created our own reference from ~400 mOSm to 1300 mOSm w/ a simple run.

Then we basically your own calibration, ie, the regression equation.

We could easily determine a standard solution, eg

1235 mOSm / 3.81% = 1500 mOSm / X X = 4.700% NaCl = 1500 mOSm $r^2 = .999$

Actually: % NaCl = 3.165E-3 (mOsm) − .050

100 mOSm = ~~0.27~~ 0.27% NaCl solution by wt.

500 mOSm = 1.53% NaCl

→ 1000 mOSm → = 3.12% NaCl

1500 mOSm 4.70% NaCl

2000 mOSm 6.28% NaCl

2500 mOSm 7.86% NaCl

3000 mOSm. 9.44% NaCl

3.12% NaCl = 96.88 gms H_2O
3.12 gms NaCl

MULTI

Tech: _____

Date: _____ Sam

ple # mOsm

1 1270

2 656

3 428

4 164 Dilute Filtered Protein

MULTI

Tech: _____

Date: _____ Sam

ple # mOsm

2 PREFREEZE Can discard and 3.87% NaCl

2 661 1.935% NaCl

3 429 1.29% NaCl

4 171 OK to Dilute Protein

6 NO FREEZE Concentrated Protein - Must Dilute

OK, we are likely to have some very usable data here.

Time to study our molecular weight determination now.

CDB Protein Analysis
Osmometry — Freezing Point Depression — mOsm Determination

F

MULTI

Tech: _____

Date: _____ Sam

ple # mOsm

2 PREFREEZE

2 664 1.935% NaCl

3 434 1.290% NaCl

Osms Concentration 4 172 Dilute Filtered Protein

5 739 Conc Protein Then Dilute. 1 part protein 4 parts H_2O

Run Complete

We also know now that the data relationship is most definitely linear, as we hoped and expected that it should be

Assuming NaCl, % = 0.49% Solution

Assuming NaCl % = 2.289% Solution
However, we have a dilution factor of 5 (1 part protein + 4 parts H_2O)
So therefore our % = 5 (2.289%)
= 11.445%

Therefore our Concentrated protein is equivalent to a 11.445% NaCl solution and the mOSm is 315.142 (11.445%) + 16.269

Concentrated Protein. = 3630 mOSm = 3.63 Osm

Now we need to see if we can back out a molecular weight estimate.

First trial on molecular mass.
Last method from ~~last~~ lab experiment PDF

Need $\frac{W_B}{W_A}$ W_B = weight of solute
W_A = wgt of solvent

And mOsm values.
We have 3 solutions of NaCl

		W_B/W_A	mOsm
3.87%	$W_B = 3.87$ gms	= .040	1235
	$W_A = 96.13$ gms		
1.935%	$W_B = 1.935$ gms	.020	640
	$W_A = 98.065$ gms		
1.290%	$W_B = 1.29$ gms	.013	414
	$W_A = 98.71$ gms		

$mOsm = 30262.3\left(\frac{W_B}{W_A}\right) + 26.62$ $r^2 = .9997$

$\left(W_B/W_A\right) = 3.3034E\text{-}5\,(mOsm) - 8.12E\text{-}4$ $r^2 = .9997$

Actual molecular wt of NaCl = 58.5 gms/mol

The intercept of the regression line can be used to determine molecular mass. We have 26.6 here. I believe however, that the no. of moles is going to be a factor here: $NaCl \rightarrow Na^+ + Cl^-$ which gives us 2 moles total.

I believe we will end up having 2(26.6) = 53.2 gms/mol vs actual of 58.5 gms/mol but all in due time we will work on this.

It is also of interest that 53.2/58.5 = 0.91 and recall the "Van Hoff factor" which you found seems to range from 1.0 to 2 in the literature for NaCl. Many interesting things to look into here. We will work it through from the beginning.

ie, what if you do not have Complete dissassociation?

Freezing Point Depression: $\Delta T_f = -K_f m_B$

K_f = Cryoscopic Constant = $1.86\,K \cdot kg \cdot mol^{-1}$
m_B = molality of solute, ie no. of moles solute/kg of solvent

Osmole is a different method of expressing concentration
An osmole is the number of moles of a compound that contributes to a solution osmotic pressure.
Osmolality is a measure of the osmoles of solute per kilogram of solvent.
(≅1000 mOSm)
A 1 Osmole solution (doesn't matter what it is) causes a freezing point depression of 1.86°C

Freez. Point Depressions

For our solutions: 3.87% → 1,235 Osm (1.86°C) = −2.30°C
1.935% → .640 Osm (1.86°) = −1.19°C
1.290% → .414 Osm (1.86°C) = −0.77°C

Therefore, mOsm (or Osm) is a direct reflection of the actual freezing point depression when multiplied by k_f. Great.

Now ya know what the instrument output actually means.

Dec 03 2018

Osmomele Trials:

Beaker 111.39
Beaker w/H_2O 204.98
Δ = 93.59 H_2O
BKr, H_2O, NaCl 210.58
Δ = 5.60 gms NaCl

Percent Solution = $\frac{5.60 \text{ gms}}{93.59 + 5.60}$
$= 5.645\%$ NaCl
$\frac{W_B}{W_A} = \frac{5.60}{93.59} = .0598$

X		NaCl				NaCl	
1 1747	1 1746	1X	NaCl	1762	+.022	1732	+.021
2 912	2 910	2X		2 926 / 914	+.010	2 938 / 911	+.031
3 611	3 612	3X		3 618 / 610	+.010	3 628 / 605	+.026
4 395	4 393	5X		4 400 / 395	.010	4 409 / 397	+.041
							X = +.030
						No time Correction 5 311	Raw Milk

Run Complete

~10 min later - evaporation

~15 min later

Evaporation/Time Correction

Milk

5	10X	100 ul + 9 (100 ul) [0.9 ml]	= 1 ml	61
6	30X	100 ul + 29 (100 ul) [2.9 ml]	= 3 ml	40
7	50X	100 ul + 49 (100 ul) [4.9 ml]	= 5 ml	38
5*	RAW			311

You melted a set of sample tubes.
Keep temp C ~ 50°C, not 125°C!

The first step was to apply the time/evaporation correction. Our final data on the salts was

	W_B/W_A	mOsm
1x	.0598	1747
2x	.030	912
3x	.020	611
5x	.012	395

This regression gives a MW estimate of 67.0

$mOsm = 28363.7\left(\frac{W_B}{W_A}\right) + 52.6 \qquad r^2 = .9999$

$W_B/W_A = 3.525E\text{-}5\,(mOsm) - 1.85E\text{-}3 \qquad r^2 = .9999$

Now this is really quite interesting. Our intercept the term is 52.6 which is quite close to the actual value of 58.5 gms/mol for NaCl.

Using Van Hoff = 1.9

Also notice our r^2 values is higher and also notice that we used 3 sets of time/evaporation corrected data. This may look made a difference. Continue w/ trials to determine the exact calculation.

The paper on milk analysis is the only one that I have found that talks of the zero intercept and the regression line as being a means to ascertain molecular weight.

These results say something for redundant data as well as the time/evaporation correction.

I think we need to repeat these trials until they settle in.

—

Now our milk data is very limited, but lets take a look @ it. Most of our data is outside the calibration range.

For raw milk, we have a $^{W_B}/_{W_A} = 1.0$ do we not.
For our 10x we have $^{W_B}/_{W_A} = 1/9 = .111$
So we have

W_B/W_A	mOsm
1.0 not really	311
.111 (1/10)	61 (tentative)

We get $mOsm = 281.2\left(^{W_B}/_{W_A}\right) + 29.8$

And the intercept makes no sense whatsoever as milk has a molecular wt 340 gms/mol.

Is there a case where the slope of the regression line does have meaning?
We need to repeat both milk & salt.

There are important discussion in the lab experiment on the differences between mixture and single solute, dissassociation or not.

We know that salt dissassociates. There is no reason to think that that milk does.

Notice what we actually have is $W_B = 1$
$W_A = 0$
for raw milk, so this is not possible to use in our regression.

There are interesting relations to look @:

$$\Delta T_f = -K_f m_B$$

K_f is the cryoscopic constant of solvent
$= 1.86°C\ kg \cdot mol^{-1}$ for water
a 1 Osm solution causes a ΔT of $-1.86°C$ if dissolved in water.

m_B is the molality of solute = moles solute / kg of solvent

We also have"

$$\Delta T_f = -\frac{K_f}{M_B}\frac{W_B}{W_A}$$

W_B = mass of solute
W_A = mass of solvent

M_B is the solute molecular mass.

I want to look @ this relation a little more . . .

The last relation looks to be very useful as we can arrange to.

$$M_B = \frac{-K_f}{\Delta T_f}\frac{W_B}{W_A}$$

only for a single solute which does not dissociate!

Now we don't actually measure ΔT_f but we do measure mOsm

How does this work? W_B/W_A is usually a small number $\frac{-K_f}{\Delta T}$ could easily be one so how do we interpret a small M_B = number?

$1\ Osm \Rightarrow -1.86^\circ C$

So $\Delta T = Osm(-1.86^\circ C)$

or $\Delta T = \frac{mOsm(-1.86^\circ C)}{1000}$ and we do measure mOsm.

So $mOsm = \frac{1000 \cdot \Delta T}{-1.86^\circ C}$

so we have

$$M_B = \frac{-K_f}{\left(\frac{mOsm}{1000}\right)(-1.86^\circ C)} \cdot \frac{W_B}{W_A} = \frac{-K_f \cdot W_B}{\frac{1}{\frac{mOsm(-1.86^\circ C)}{1000}} W_A}$$

$$M_B = \frac{-K_f \cdot W_B \cdot 1000}{mOsm(-1.86^\circ C) W_A}$$ but $K_f = 1.86^\circ C$ So:

$$M_B = \frac{W_B \cdot 1000}{W_A \cdot mOsm}$$ $\frac{kg}{mol}$!!!

only for a single solute that does not dissociate!

How do you interpret this?

Let's look @ some other sources and redo
also clarify units before proceeding.

K_f is in units $\frac{°C \cdot kg}{mol}$ $\quad$ $\frac{W_B}{W_A}$ is a ratio so it is unitless.

ΔT is in C° so we are left w/ kg/mol
What is why the number is so small.
So we multiply by 1000 to get molecular mass in gms/mol

which is what we needed to know.
Understand the relation is only for a single solute that does not dissociate.

Also we see that we can apply that to
a serum reading.

Notice if we apply that to our diluted protein yesterday (1 to 4 ratio)

We end up with the following, assuming it does not dissociate:

$$\frac{M_B}{(kg/mol)} = \frac{200\,\mu l \text{ protein}}{800\,\mu l\ H_2O} \cdot \frac{1000}{mOsm} = \frac{0.25\,(1000)}{739\ mOsm}$$

(or 1 to 4 parts no matter how you do it)

$$= 0.338\ kg/mol$$

$$= \frac{338\ gms}{mol}$$

Very, very interesting. This is a reasonable number.

(This is in the range of milk).

This says that all we actually need to solve for the molecular weight of a non dissociating solute is:

1. the dilution ratio
2. the mOSm reading

] this is quite amazing, nothing about the protein otherwise is known.

This would be quite astounding. Salt is certainly not in this category but the protein might be.

Furthermore

Since we have $M_B = \frac{W_B}{W_A} \cdot \frac{1000}{mOSm}$ in $\left[\frac{kg}{mol}\right]$

We know that M_B (in gms/mol) $= \frac{W_B}{W_A} \cdot \frac{1000}{mOSm} \cdot 1000 \Rightarrow M_B \text{ (gms/mol)} = \frac{W_B}{W_A} \cdot \frac{1000}{mOSm}$

So now we have $M_B \text{ (gms/mol)} = \frac{\left(\frac{W_B}{W_A}\right) \cdot 1000^2}{mOsm}$ or $M_B \text{ (gms/mol)} = \frac{\left(\frac{W_B}{W_A}\right) \cdot 1E6 \cdot n}{mOSm}$

and we also see that this is going to be multiplied by n the number of particles it dissociates into.

Van Hoff of 1.9 as referenced in literature to the last row.

Regression Intercept $MW = a\left(\frac{WB}{WA}\right) + b$ ↑ $b = 58.0$ $r^2 = .77$

Look @ our menu for salts, there is:

		(1.8?)	$n=2$ (1.9)	$n=1.8$
34.2	$MB = \frac{.0590 \cdot 1E6 \cdot 2}{1747}$		68.5	65.0	61.6
32.9	$\frac{.03(1E6)}{912}$		65.8	62.5	59.2
32.7	$\frac{.02(1E6)}{611}$		65.5	62.1	58.9
30.4	$\frac{.012(1E6)}{395}$		60.8	57.8	54.7
			$\bar{x} = 65.2$		$\bar{x} = 58.6$

Actual Value for NaCl is 58.5 vs 58.6 !!

I think that it is time to recall the Van Hoff factor !!! It was not 2, it was 1.8

Ok, we are definitely on the right track here.

There is an ideal dissociation factor and there is an actual dissociation factor, determined by experiment only.

Non ionizing solutions have a factor of 1 both real and ideal.

Van Hoff Factor - Important

219A

Osmosis Equation

Return to Solutions Menu

Go to Reverse Osmosis

Go to Osmosis

NaCl
$i = 1.8$, not 2.0 ↘ ideal
↘ actual

The osmosis equation is:

$$\pi = iMRT$$

π is not equal to 3.14159 in this situation. π stands for the osmotic pressure and is usually expressed in the pressure unit of atmospheres.

The definition of osmotic pressure: the amount of pressure required to stop the process of osmosis in your experimental set-up.

The lowercase letter "i" is called the van 't Hoff factor and it will be dealt with in the problems below. It is named for Jacobus Henricus van 't Hoff (Henry to his friends), who applied PV = nRT to solutions and figured out why "i" was needed and what it represents. The image just to the right is a 23K GIF of him.

He was awarded the first Nobel prize in chemistry in 1901 and the ChemTeam thinks this is the official portrait selected from the many pictures taken at the photo session. Love that hair! From the late 1870's to the turn of the century, van 't Hoff was one of the premier chemists in the world.

M is molarity: good old moles per liter.

R is the gas constant and we will be using the same value as in the gas laws unit: 0.08206 L atm/mol K. Now, you may ask what a "gas" constant is doing in a discussion of solutions. Well, for one thing it's called the "gas" constant because it was discovered in the course of research on gases.

Also, van 't Hoff's insight was to see that PV = nRT applied to molecules of solute moving though the solvent. (There is an article called How the Theory of Solutions Arose, which is about his insight. It is in the Classic Papers section of the ChemTeam.) In essence, the molecules of solute are a "gas," dispersed through the "universe" of solvent molecules. If I were to move V to the right side, I would get:

$$P = (n / V)RT$$

(n / V) is moles divided by liters and that is molarity.

T is temperature, measured as usual in Kelvins.

What is the osmotic pressure of a 1.00 M solution of sucrose at 25°C?

When we insert into the equation, we have:

$$\pi = i\ (1.00\ mol/L)\ (0.08206\ L\ atm / mol\ K)\ (298\ K)$$

2 19 B

However, there are two unknowns: π, the one we want and i. What is i?

Once again, i is called the van 't Hoff factor.

The van 't Hoff factor is a unitless, empirical constant related to the degree of dissociation of the solute.

WHAT IN THE WORLD DID HE JUST SAY???

OK, OK. The value is unitless. That means it is just a number like 1 or 2. Empirical means we must determine it by experiment. You can predict what a theoretical value for i might be, but the real value is only found in an experiment. The explanation follow shortly as to why.

The key is "degree of dissociation." This refers to the fact that some molecules ionize in solution (they split into their positive and negative ions) and other do not. This idea was put forth by Svante Arrhenius in 1884 in his Ph.D. dissertation and it was soundly rejected. (Arrhenius on electrolytic dissociation links to an excerpt from his article which announced this concept to the world.) In 1903, he was awarded the Nobel Prize in chemistry for it. Today, it's part of the common ordinary high school chemistry curriculum.

The van 't Hoff factor for sucrose is 1, since sucrose does not ionize in solution. It remains as whole molecules.

So the answer is 24.4 atm.

What is the osmotic pressure (at 25°C) of seawater? It contains approximately 35.0 grams of NaCl per liter. (Seawater contains other stuff, but we'll ignore it.)

Convert grams to moles:

35.0 g/L ÷ 58.443 g/mol = 0.599 mol/L

Now, plug into the equation:

$\pi = (i)\ (0.599\ mol/L)\ (0.08206\ L\ atm / mol\ K)\ (298\ K)$

There's that pesky van 't Hoff factor. What is its value for NaCl?

When NaCl ionizes in solution it produces Na^+ ions and Cl^- ions. One mole of NaCl produces 1 mole of each type of ion. So the van 't Hoff factor is, theoretically, equal to 2. However, we will use 1.8 and I'll explain that in a moment.

So, plug again and then solve:

$\pi = (1.8)\ (0.599\ mol/L)\ (0.08206\ L\ atm / mol\ K)\ (298\ K)$

$\pi = 26.4$ atm

Why did I use 1.8 for the van 't Hoff factor for NaCl rather than 2?

This has to do with a concept called ion pairing. In solution, a certain number of Na^+ ions and Cl^- ions will randomly come together and form NaCl ion pairs. This reduces the total number of particles in solution, hereby reducing the van 't Hoff factor.

Go to Reverse Osmosis

Go to Osmosis

2 / 9 C

13.9: SOLUTIONS OF ELECTROLYTES

- Page ID

Thus far we have assumed that we could simply multiply the molar concentration of a solute by the number of ions per formula unit to obtain the actual concentration of dissolved particles in an electrolyte solution. We have used this simple model to predict such properties as freezing points, melting points, vapor pressure, and osmotic pressure. If this model were perfectly correct, we would expect the freezing point depression of a 0.10 m solution of sodium chloride, with 2 mol of ions per mole of \(NaCl\) in solution, to be exactly twice that of a 0.10 m solution of glucose, with only 1 mol of molecules per mole of glucose in solution. In reality, this is not always the case. Instead, the observed change in freezing points for 0.10 m aqueous solutions of \(NaCl\) and KCl are significantly less than expected (−0.348°C and −0.344°C, respectively, rather than −0.372°C), which suggests that fewer particles than we expected are present in solution.

The relationship between the actual number of moles of solute added to form a solution and the apparent number as determined by colligative properties is called the van't Hoff factor (*i*) and is defined as follows:Named for Jacobus Hendricus van't Hoff (1852–1911), a Dutch chemistry professor at the University of Amsterdam who won the first Nobel Prize in Chemistry (1901) for his work on thermodynamics and solutions.

$$i=\frac{\text{apparent number of particles in solution}}{\text{ number of moles of solute dissolved}} \tag{13.9.1}$$

NOTE

As the solute concentration increases the van't Hoff factor decreases

The van't Hoff factor is therefore a measure of a deviation from ideal behavior. The lower the van't Hoff factor, the greater the deviation. As the data in Table 13.9.1 show, the van't Hoff factors for ionic compounds are somewhat lower than expected; that is, their solutions apparently contain fewer particles than predicted by the number of ions per formula unit. As the concentration of the solute increases, the van't Hoff factor decreases because ionic compounds generally do not totally dissociate in aqueous solution.

Table 13.9.1: van't Hoff Factors for 0.0500 M Aqueous Solutions of Selected Compounds at 25°C

Compound	i (measured)	i (ideal)
glucose	1.0	1.0
sucrose	1.0	1.0
\(NaCl\)	1.9	2.0
\(HCl\)	1.9	2.0
\(MgCl_2\)	2.7	3.0
\(FeCl_3\)	3.4	4.0
\(Ca(NO_3)_2\)	2.5	3.0
\(AlCl_3\)	3.2	4.0
\(MgSO_4\)	1.4	2.0

Some sources give 1.8 → see following reference

Instead, some of the ions exist as ion pairs, a cation and an anion that for a brief time are associated with each other without an intervening shell of water molecules (Figure 13.9.1). Each of these temporary units behaves like a single dissolved particle until it dissociates. Highly charged ions such as \(Mg^{2+}\), \(Al^{3+}\), \(SO_4^{2-}\), and \(PO_4^{3-}\) have a greater tendency to form ion pairs because of their strong electrostatic interactions. The actual number of solvated ions present in a solution can be determined by measuring a colligative property at several solute concentrations.

219 D

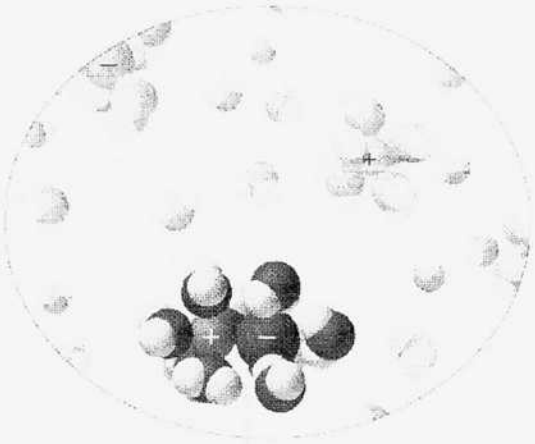


Figure 13.9.1: Ion Pairs. In concentrated solutions of electrolytes like \(NaCl\), some of the ions form neutral ion pairs that are not separated by solvent and diffuse as single particles.

EXAMPLE 13.9.1: IRON CHLORIDE IN WATER

A 0.0500 M aqueous solution of \(FeCl_3\) has an osmotic pressure of 4.15 atm at 25°C. Calculate the van't Hoff factor \(i\) for the solution.

Given: solute concentration, osmotic pressure, and temperature

Asked for: van't Hoff factor

Strategy:

A. Use Equation 13.9.12 to calculate the expected osmotic pressure of the solution based on the effective concentration of dissolved particles in the solvent.

B. Calculate the ratio of the observed osmotic pressure to the expected value. Multiply this number by the number of ions of solute per formula unit, and then use Equation 13.9.1 to calculate the van't Hoff factor.

Solution:

A If \(FeCl_3\) dissociated completely in aqueous solution, it would produce four ions per formula unit [Fe3+(aq) plus 3Cl−(aq)] for an effective concentration of dissolved particles of 4 × 0.0500 M = 0.200 M. The osmotic pressure would be

$$\Pi=MRT=(0.200\;mol/L)\left[0.0821\;(L\cdot atm)/(K\cdot mol)\right](298\;K)=4.89\;atm$$

B The observed osmotic pressure is only 4.15 atm, presumably due to ion pair formation. The ratio of the observed osmotic pressure to the calculated value is 4.15 atm/4.89 atm = 0.849, which indicates that the solution contains (0.849)(4) = 3.40 particles per mole of \(FeCl_3\) dissolved. Alternatively, we can calculate the observed particle concentration from the osmotic pressure of 4.15 atm:

$$4.15\;atm=M\left[0.0821\;(L\cdot atm)/(K\cdot mol)\right](298\;K)$$

$$0.170\;mol/L=M$$

The ratio of this value to the expected value of 0.200 M is 0.170 M/0.200 M = 0.850, which again gives us (0.850)(4) = 3.40 particles per mole of \(FeCl_3\) dissolved. From Equation 13.9.1, the van't Hoff factor for the solution is

$$i=\frac{\text{3.40 particles observed}}{\text{1 formula unit}\;FeCl_3}=3.40$$

EXERCISE 13.9.1: MAGNESIUM CHLORIDE IN WATER

Calculate the van't Hoff factor for a 0.050 m aqueous solution of \(MgCl_2\) that has a measured freezing point of −0.25°C.

Answer: 2.7 (versus an ideal value of 3

KEY CONCEPTS AND SUMMARY

Ionic compounds may not completely dissociate in solution due to **activity** effects, in which case observed colligative effects may be less than predicted.

We are now showing fantastic results.

Let's look again @ what we have for milk.

Our developed equation is

$$M_B = \frac{\left(\frac{W_B}{W_A}\right) \cdot 1E6 \cdot \text{"n"}}{mOsm}$$

(Van Hoff factor is called "i")

We simply do not have good values for milk yet.

We need to try 2x, 3x, 4x.

We have our good measurement for the protein.

Question, is it ionic? Not expected to be.

We have one case where $\left(\frac{W_B}{W_A}\right) = 0.25$

We have a measured mOsm of 739

Therefore our first estimate of the CDB protein (soluble) molecular wt is

$$= \frac{0.25 \cdot 1E6 \,(1)}{739} \quad \text{(1 presumed)}$$

$$= 338 \frac{gms}{mol}$$

* important first estimate

1 Dalton = 1 gm/mole.

1 KDa = 1000 gms/mole

We are @ ~ 0.34 KDa = 338 Da.

An average amino acid has a molecular mass of 100 Da.

Lactose is 342 gms/mole $C_{12}H_{22}O_{11}$
Albumin is 66.5 kdA
Casein (milk) a protein, is on the order of 784 gms/mol

Glutathione is 307 gms/ml
This is the smallest functional polypeptide w/ only three amino acids.

This looks to be our neighborhood.

We need to start regularly treat a bath mill and our protein.

The average size of an amino acid is 110 Da.
We therefore are almost certain to have 3 amino acids involved, having a strong analogy to the glutathione situation.

Now, there is an element of conductivity to the harvested soluble protein

However we seriously have to wonder if the cows from various countries in the culture medium such as Fe, Cl, salt, potato ions, etc

The would almost certainly alter the case. Here is the question:

Is <u>any</u> protein known to be ionic?

Charged amino acids can form ionic bonds but these are not ions.

Usually globular proteins are soluble, fibrous ones are not. Denaturation changes the structure & the proteins no longer globular.

Hydrophylic side chains in the protein will be exposed. The hydrophylic side chains will be on the outside of the protein.

But they <u>don't dissociate</u>.

So we are likely dealing with a very small (3 amino acids), globular, with hydrophylic side chains.

*

Haemoglobin is a globular protein as an example.
Amylase (saliva) is also globular
Globular proteins play many biological roles.

Act as 1. enzymes
2. hormones
3. immunoglobins
4. transport molecules
} as examples

Proteins are either fibrous or globular
(Guess what? we have both)

Fibrous – strength & structure
Globular – move around in fluids compact so they can be inserted into cell membranes
(eg membrane transport proteins, enzymes, neurotransmitter receptors, antibodies. -)

Current Brix of Protein is 30.0.
Lets investigate the protein @

3x 1000 ~~200~~ ul Protein , 2 ml H_2O

4x 1000 ul Protein 3 ml H_2O

7x 1000 ul Protein 6 ml H_2O

10x ~~1000~~ 1000 ul Protein 9 ml H_2O

Protein
Questions
Here

Set C

VOLTI

Sam

(335)

(650)

3 8[illegible]

No need to remove Outliers scanning

OK, we have some difficulties in acquiring consistent data across all dilutions, but we appear to have usable data.

w_B/w_A	mOsm	$\bar{X}$
1/82	835, 815, 856	835
1/43	650, 619, 605	625
1/16	384, 416	400
1/09	310, 330	320

∴ $r^2 = .9995$ OK good

Regression results are of interest,

$mOsm = 1323.0\left(\frac{w_B}{w_A}\right) + 177.5$ $r^2 = .9995$

Molecular weight determination

$$M_B = \frac{\left(\frac{W_B}{W_A}\right) IEG \cdot (1)}{mOsm}$$

	Dilution	MW Determination	
1/2	~~3x~~	$\frac{(.5)\,IEG\,(1)}{835} = 599$	1791
1/3	~~4x~~	$\frac{(1/3)\,IEG\,(1)}{625} = 533$	2133
1/6	~~7x~~	$\frac{(1/6)\,(IEG)\,(1)}{400} = ~~354~~\ 417$	2919
1/9	~~10x~~	$\frac{(1/9)\,(IEG)\,(1)}{320} = 347$	

Tentative $\bar{X} = 386$

The data is certainly more variable than we would like. Our most reliable data appear to be with the lower dilutions.

I think we need to readjust our run to

2x	W_B = 1 ml	W_A = 1 ml
3x	"	2 ml
4x	"	3 ml
5x	"	4 ml

Ok, this run on the protein looks much better. One lesson you have learned is to keep the dilutions as low as possible that still allow easy freezing.

You also learned about this w/ your ice baths. Your best work came when the ratio of WB/WA is as high as can feasibly be made.

MULTI

Tech: _____

Date: _____ Sam

	pie #	mOsm
2x	1	1258
3x	2	782
4x	3	572
5x	4	470

WB/WA	mOsm	MW
1/1	1258	795
1/2	782	639
1/3	572	583
1/4	470	532

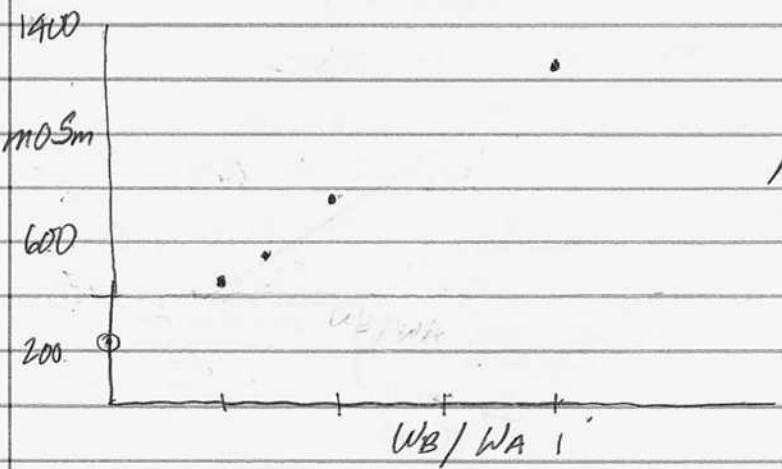
OK I do not believe the method is correct in the case of a mixture. Let's look @ regression again

$mOSm = 1039.63\left(\frac{W_B}{W_A}\right) + 229.0 \qquad r^2 = .996$

$\left(\frac{W_B}{W_A}\right) = 9.57ME{-}4(mOSm) - .217 \qquad r^2 = .996$

I strongly suspect the slope of the regression, ie. 1039.6 is our estimate for the MW.

Our lowest dilution give our best result. I suspect it will converge at the slope. Not sure why yet



$y = ax + b$

$MOSm = a\left(\frac{W_B}{W_A}\right) + b$

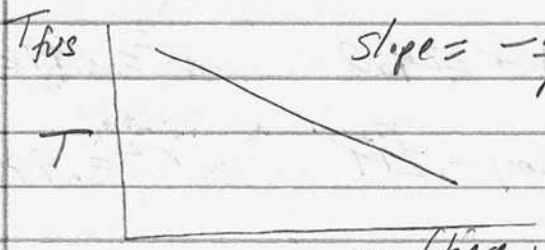
$\frac{d(mOSm)}{d\left(\frac{W_B}{W_A}\right)} = a$

Notice on our previous run, the regression is

$mOSm = 1323.0\left(\frac{W_B}{W_A}\right) + 177.5 \qquad r^2 = .9995$

Notice these also in similar range. Mean slope = 1182
Mean intercept = 203

We have to wonder if these the realistic estimate for MW. Study the regression theory if you can find it. Our paper does not explain where these come from.



& do first another page that shows molecular wt being determined from regression slope but they do have a different relationship.

Assume:

$$\text{Slope} = \frac{-K_f}{m_B} \quad \text{or} \quad m_B = -\frac{K_f}{\text{slope}}$$

$K_f = 1.86 \frac{^\circ C\ kg}{mol}$ slope, in this case =

$$\frac{^\circ C}{\frac{\text{mass in kg}}{1\ kg}}$$

so $\frac{^\circ C \cdot kg}{mol}$ $\left(= C \cdot kg \left(\frac{1\ kg}{\text{mass in kg}}\right) \frac{}{^\circ C \cdot mol}\right)$

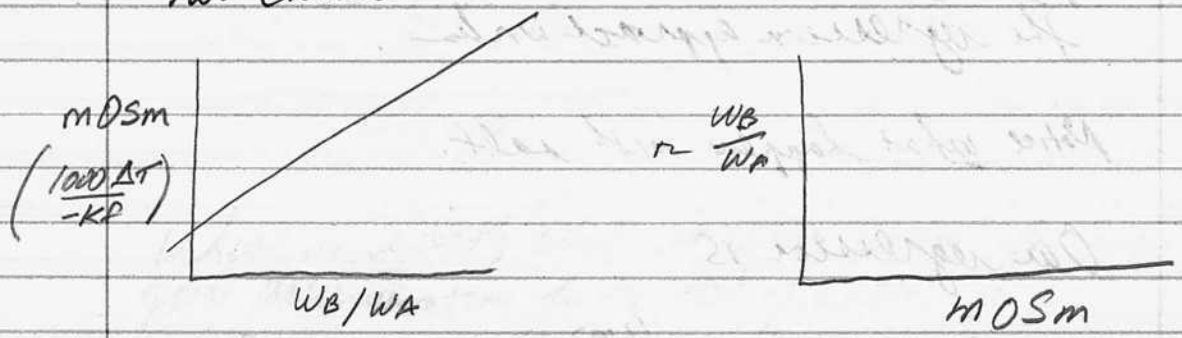
$$\frac{C}{\left(\frac{\text{mass in kg}}{1\ kg}\right)}$$

reduces to $\frac{\text{mass in kg}}{mol}$ and therefore is the molecular weight in kg/mol (which we can multiply by 1000 to get gms/mol)

OK

Idea, for the first time shows us how the slope of the regression line can be used to determine the molecular wt of wvl based the correct functional relationship.

Now let's start looking @ what we have ..
Two choices



We know that $mOSm = \frac{1000\Delta T}{-K_f}$

So slope for vs is: $\frac{\Delta \frac{1000\Delta T}{-K_f}}{\Delta\ W_B/W_A}$ $= \frac{{}^\circ C \cdot 1000}{{}^\circ C \cdot kg / mol}$ $\left(= \frac{{}^\circ C \cdot mol \cdot 1000}{1\ {}^\circ C \cdot kg}\right)$

(ratio which is unitless)

and here we see that the inverse of our slope needs to be taken and multiplied by 1000 to get gms/mole and then by another 1000 because of Constant. $\left(\frac{1}{1039.63}\right) 1E6 = 962$ gms/mole

Previous regression $\left(\frac{1}{1323}\right) 1E6 = 756$ gms/mole

This run will be disregarded highly deviated

$X = 859$ gms/mole

This is actually our current estimate.

Now, notice MW for each point of the NaCl problem was all the same,

where MW of the protein is varying @ each point of computation.

Let's look @ the salt cases and see how the regression approach works.

Notice what happens with salt.

Our regression is

$$mOSm = 28363.7\left(\frac{W_B}{W_A}\right) + 52.6 \qquad r^2 = .9999$$

Now if we take the inverse of this slope * 1E6 we get 35.2

If we apply the Van Hoff factor we get
$35.2(1.6) = 63.8$ gms/mol vs 58.5

So the two are in range.

And even more interesting, look at your computations

We have $M_B = \frac{(W_B/W_A)\cdot 1E6}{mOSM}$

Which is exactly the inverse of our slope from our functional relationship · 1E6 !!!

They are doing the same thing!

mOSm | Guess what the inverse of this slope is · 1E6

WB/WA

$\left(\frac{WB}{WA}\right) \cdot 1E6 \cdot \frac{1}{mOSm} \cdot L$

Van Hoff factor

Which is exactly what we were using for our point determination for the MW of salt.

Interesting that for NaCl, each individual point computation (and especially the average) came out right on target.

Notice that the regression approach, ie inverse of slope · 1E6 · Van Hoff factor comes out a little high, not sure why, but we expect the regression approach to generally be preferred since it is easier in application.

It looks like we are now led to a common point of method of molecular weight determination.

1. The average of numerous regressions should give us the best result.

2. Keeping the dilution factors low (ie, high mOSm) should also give us improved values.

Let's look @ some error analysis.

Our slope result is based upon

$$y = \frac{X \cdot a}{z}$$

$$\frac{dy}{dx} = \frac{a}{z} \quad \& \quad \Delta y = \frac{a}{z}\Delta x$$

$$\Delta y = -(X \cdot a) z^{-2} \Delta z \qquad X = {}^{WB}/_{WA}$$

$$= -\frac{X \cdot a}{z^2}\Delta z \qquad z = MOsm$$

$$a = 1E6$$

The derivation the inverted slope w.r.t. the mass ratio increases when the error in the mass ratio changes. As a percentage, this error will be greater when M_B is small.

Also the larger the value of mOsm the smaller the error will be.

This agrees w/ the hypothesis that lower dilution ratios are advantageous to reduce error.

Now, the error in the inverse slope w.r.t. an error in MOSM will be much smaller when the MOSM is large. The influence reducing error from a high MOSM will be greater than that of an increased weight ratio because of the squared term.

Agreed, the lowest deletion ratio achievable (ie, with freezing and ≤ 2000 MOSM from the instrument)

and the highest MOSM w/in the achievable limits of the instrument will reduce the error in determination of the inverse slope.

The further confirms to us that our most recent freezing point depression value is likely our best estimate then for of the molecular wt (MW) of the protein.

MW estimate (gms/mol) is currently:

962 gms/mole ≅ 1 KdA

Therefore we see currently that our bands in the protein mw determinations are expected to be close to 962 gms/mol due to the improved dilution factors used.

We could even try a ratio of

½ X 0.5 ml solute or the next dilution.
     1.5 ml H_2O

Looks like you are zeroing in on the method.

Phosphate treated culture observation:

Many "cellular" unit formation on the surface of the liquid of the small scale phosphate treated cultures. Photographed @ 200x.

In general, phosphate treated cultures appear to be developing well, including the large scale 10" x 10" culture under development for the first time.

Regardless of whether phosphate is added or not, both culture styles seem to benefit from stirring the developed growth approx 2 times per day along w/ the heat incubation provided (heating pad)

The give the organism a fresh surface to adhere too and promote further growth of already existing protein conglomerates - filament networks continued.

— + filtered

Also the HEPA aerated culture (agar) with the "film layer drops" is now developing a full filament network. It took about one full week for the process to begin.

All agar cultures are active w developing filament networks now w/ no dry cultures.

Current Brix of concentrated protein @ 28.5
Protein Osmotic Trial
Lets set ratios as:

Protein	H_2O	Protein	H_2O
~~0.5~~ void	1	500 ul	1000 ul
1	1	1000 ul	1000 ul
1	2	1000 ul	2 ml
1	3	1000 ul	3 ml
1	4	1000 ul	4 ml

Will not Cannot this →

I am trying a trial w/ 50 ul instead of 30 ul.

Mixing of solutions is important

OK, we appear to have three very good runs
Ya apparently cannot use and W_B/W_A ratio < 1.
It will generate Monroe data.

It is also important to control evaporation in all aspects, including the original sample containers, as well as during the run, as well as to correct for trivial evaporation effects during the run themselves.

Soluble Protein Run for Molecular Weight

Run Complete

Set t=0

MULTI

Tech: _____

Date: _____ Sam

ple # mOsm

1 ~~7~~ Null

2 1187

3 739

4 564

Ok to leave in place for scanning

Teme correct the data given.

Our corrected data is

W_B/W_A	mOsm
1/1	1189
1/2	741
1/3	561

Regression:

$mOsm = 931.4\left(W_B/W_A\right) + 261.1$

$r^2 = .9984$

$MW\ estimate = \left(\frac{1}{931.4}\right) \cdot 1E6 = 1073.6\ gms/mol$

This is now a smart process, isn't it?

Our values are: 962 gms/mole
1073.6 gms/mole

$\bar{X} = 1018\ gms/mole$

Good work. Repeat.

50 ul appears to be adequate enough experiment.
Very stable results.
Mixing sample is important.
Time / Evaporation correction is important.

Ok, we have another run:
There could be some evaporation from samples.
Data set is: (Data not appears significant)

W_B/W_A	mOSm
1/1	1229
1/2	733
1/3	565

Regression:
$MoSm = 995.1 (W_A/W_B) + 234.2$

$r^2 = .99999$

Molecular weight estimate $= \frac{1}{995.1} (1E6) = 1004.9$

Continuing Molecular Wt Estimate for Soluble Protein

1 1225

3 NO FREEZE

3 565

Run Complete

MULTI

Tech: _____

Date: _____ Sam

ple # mOsm

X = +.019

+.021 1 ~~1251~~ ~~1225~~ 1228

2 ~~745~~ ~~750~~ 731

+.016 3 574 563

P

Ok, we have 3 values now:

.996 962 gms/mole

.9984 1073 gms/mole

.99999 1005 gms/mole

The straight average is 1013.3 gms/mole

But I prefer to use a weighted avg using r^2 values.

$$\frac{.996(962) + .9984(1073) + .99999(1005)}{.996 + .9984 + .99999}$$

$$= \frac{3034.425}{\Sigma w_i} = 1013.4$$

and we get the same results.

Look @ weighting factor a little more.

$1 - .996$	$= .004$	400
$1 - .9984$	$= .002$	200
$1 - .99999$	$= .00001$	1

There are ratios of difference.

		Reciprocal
$\sqrt{400} = 20$	$\sqrt[3]{400} = 7.4$	.135
$\sqrt{200} = 14$	$\sqrt[3]{200} = 5.8$	.172
$\sqrt{1} = 1$	$\sqrt[3]{1} = 1$	1
	$\sqrt[5]{400} = 3.3$	.303
	$\sqrt[5]{200}$ 2.9	.345
	$\sqrt[5]{1}$ 1	1

Use these wts.

$$\frac{.303(962) + .345(1073) + 1(1005)}{\Sigma w_i} = 1011.3$$

OK

OK, we have stability being achieved in our runs now.

Our best estimate for the molecular weight of the soluble protein is now 1011 gms/mole = 1.011 kDa

This is very close to both the simple average as well as a reasonably weighted average

OK, let's go again, from scratch. A very clean run, let's all what we have Time correct the data first.

W_B/W_A	$M_{0.5n}$
1/1	1424
1/2	899
1/3	644
1/4	522

Remember the protein is slowly more concentrated as the sperm run

$M_{0.5n} = 1185.9 (W_B/W_A) + 254.6$

$r^2 = .993$

$\frac{1}{1185.9}$ (HEG) = 843.2 Molecular Wt Estimate

OK, we definitely have more variation than we would like to see now.

All values are:

Slope					w_i: $1/w_i$ $\sum$	
			$1-\Delta$	Error Ratio		
1323	756	$r^2 = .9995$	.001	100	2.5	.40
1040	962	.996	.004	400	3.3	.30
931	1074	.9984	.002	200	2.9	.34
995	1005	.99999	.00001	1	1	1
~~841186~~	843	.993	.007	100	3.7	.27

Weighted average $= \frac{.40(756)+.30(962)+.34(1074)+1(1005)+.27(843)}{\sum w_i}$

$= 948$ nanograms/mol. OK, that knocked it down a lot.

We probably need to go again.

$\sigma = 324.9$ $\quad \sigma_{\bar{x}} = \frac{\sigma}{\sqrt{N}} = \frac{324.9}{\sqrt{5}} = 145$

That's pretty high, our expected error is a good 10^{20} you need to do this again until it stabilizes.

Date: Sam

Sample # mean

$\bar{x} = +.028$

+.010	1	1460	1420	
+.031	2	922	897	
+.041	3	666	648	
+.029	4	536	521	

Complete

Can be left in place for scanning

Next run

W_B/W_A	mOSm
1/1	1448
1/2	912
1/3	639
1/4	515

$mOSm = 1227.4(W_B/W_A) + 239.2$

$r^2 = .9905$

I can see that a power regression offers a slightly improved regression curve fit than what does not actually mean vs theoretical?

$MW_{estimate} = \frac{1}{1227.4}(1E6) = 814$

Now an interesting question is what if we disregard the lowest dilution, which we know introduces more error?

What you actually have here is a polypeptide moreso than a protein.

The decrease the slope to 1181 w/ r^2 = .9909

The leads to a MW estimate of $\frac{1}{1181}(1E6) = 847$

Same on previous run:

The would decrease the slope to 1142 w/ r^2 = .993

MW estimate is: 876

There is a reasonable basis for doing this as you can see that the weakest chlorid increase the departure from linearity and you know that it introduced more error.

This alters each data set to:

	MW	r^2	Δ	Error Ratio	$\sqrt[5]{(W_i}$	
~~Should be removed~~	~~750~~	~~.9995~~	~~.005, .0005~~	~~50~~	~~2.2~~	~~.46~~
	962	.996	.004	400	3.3	.30
	1074	.9984	.0016	100	2.8	.36
	1005	.99999	.00001	1	1	1
	876	.993	.007	700	3.7	.27
	~~814~~ 841	.991	.009	900	3.9	.26

~~2130~~ ~~1936.4~~ ~~2284.1~~

MW estimate = $\frac{2.13}{2.19} = \frac{\sim 2416.16}{2.65} \approx \sim 938$ gms/mole

916

$\sigma_{\bar{x}} = \frac{\sigma}{\sqrt{N}} = \frac{\sim 83.2}{\sqrt{5}} = \pm 37.2$ gms/mol

Corrected

~~Or to leave in place~~

~~Allowing I can~~ .

The smallest peptide ladder I can find ranges from 1.7 to 40 kDa with 6 divisions.

The mean expected error is ~ +/- 3 kDa

and you are within ~ 0.1 kDa

I would say the work here is actually pretty tight.

Weighted standard error of the mean is always an interesting question.

OK to leave in place during scan

+.002 1 1466 1444

[illegible]plete

MULTI

Tech: [illegible]

[illegible]te: [illegible] Sam

[illegible] # mGsm [illegible]

+.002 1 1463 [illegible]

2 907

The first run should be, and now is deleted. The was early on the process w/ highly dilute solution.

Our last 5 runs can be, and should be used as our basis.

The leads to the following current data set

MW	r^2	w_i
962	.996	.30
1074	.9984	.36
1005	.99999	1
876	.993	.27
841	.991	.26

Which leads to a weighted average of

MW estimate = 976 gms/mole

σ of this data set is 83.2
estimated standard error of the mean is

$$\sigma_{\bar{x}} = \frac{\sigma}{\sqrt{N}} = \frac{83.2}{\sqrt{5}} \approx 37 \text{ gms/mole}$$

These are very reasonable numbers.
We seem to be dealing w/ a ~ 1 kDa polypeptide

i.e., an extremely small globular, water-soluble protein.

If you recall, your earlier ones were more prone to evaporation loss so they could have resulted in higher concentration levels. The number looks very reasonable with all things considered.

Another run.

Mixed solutions well, controlled evaporation, temp corrected.

W_B/W_A	mOsm
1/1	1351
1/2	807
1/3	615

$mOsm = 1100.3\left(W_B/W_A\right) + 252.0$

$r^2 = .99986$

$MW\ estimate = \frac{1}{1100.3}(1E6) = 908.9$

Current Data Set

		A	W_1
962	.996	.004	.30
1074	.9984	.0016	.36
1005	.99999	.00001	1
876	.993	.007	.27
841	.991	.009	.26
909	.99986	.00014	.59

$\bar{X}_W = \frac{2673.3}{2.78} = 962$ gms/mole

$\sigma = 77.1$ $\quad \sigma_{\bar{X}} = \frac{77.1}{\sqrt{6}} = 32$

Ok, we appear to have very good & consistent data @ this point.

This should be sufficient for the project.

Molecular weight estimate for soluble protein (~~pept~~ i.e. (polypeptide) is

$MW \cong 962$ gms/mole

$\sigma_{\bar{x}} = 32$ gms/mole (3.3%)

Very decent numbers here.

We anticipate a chain of ~ 9 amino acids (not necessarily distinct) to make up the polypeptide.

We will next confirm our methods w/ Strep on milk and/or vegetable juice.

Good job thus far.

Ok to leave during scan.

Run Complete

MULTI

Tech: _____

Date: _____ Sam

ple # mOsm

1 1353

2 803

3 617

Run Complete

MULTI

Tech:

Date: Sam

Anti bacterial polypeptides (AMP's) are a microbial very hot topic of research. Usually 12-50 amino acids

Toxic → Protein toxins produced by bacteria can be used for cellular targeting, eg against tumors.

Antimicrobial Polypeptides, also in reverse are, or can be a major research lead.

energy possibilities
biodegradable plastic possibilities

Junior Projects now include:

1. Verify osmolality methods w/ milk
2. Media for cultures & stored proteins
3. DNA prospects
4. Skin Examination Project
5. Toxicology Project
6. Legacy – index notebooks

Dec 05 2018 Page 249

Now let's look @ milk as a comparison. Our mOSm value obtained are very low, it is borderline on data collection. Apparently ≤ 90 is outside calibration range but you can at least write down the value obtained from the panel.

Time correct the data.

WB/WA	mOSm
1/1	153.5
1/2	106
1/3	88.5

Leave in place during scan

$Mosm = 96.9194 (WB/WA) + 56.77$
$r^2 = .9996$

The inverse slope of this is 10318 gms/mol.
$\frac{1}{96.9194}$ (IEC) $= 10318$.

Very interesting results.
Closest seems to be lactoferrin @ ~ 14 KDa (we have 10.3 KDa).
Caseins, however are 25-35 KDa.

You also seem to be near the limits of detection on the instrument so there may be caseys on issue in detection as well.

There are a series of proteins in milk.

Caseins 25-35 kDa
Whey 18 kDa
Lactalbumin 14 kDa ← This is our closest.
Lactoferrin 80 kDa
Serum albumin 66 kDa

We can certainly see that our detection is entirely different from that of the soluble proteins.

Or, we do have a level of compensation. The osmolality of whole milk is ~ 300 mOsm per kg.

This is different where we are.

Since our milk is diluted at least by a factor of 1 to 1, we expect whole milk to have an mOsm of ~ 306

which matches quite closely.

Let's try whole milk.

mOsm

1 304

Whole Milk has come → out spot on projection

Milk Osmolity

If a little's good, is more better?

Mixing milk replacers for winter feeding of dairy calves

Page 251 A

Dale Moore, Helen Floren and William Sischo for *Progressive Dairyman*

The total nutrients consumed by pre-weaned calves affects their average daily gain (ADG). What is even more intriguing is some evidence that the calf's ADG before weaning impacts its first-lactation milk yield. At Cornell, calves on two farms in New York were studied. For every 1-pound increase in ADG, the heifers, on average, produced 841 pounds more milk in their first lactation. Those born during winter produced about 1,200 pounds less milk in their first lactation compared to calves born during the summer. Increasing calves' nutrient intake in the winter not only gives them additional energy for their higher maintenance needs, it also improves their future milk production.

Most producers are aware that in cold climates, calves need more nutrients in the winter. Adding more milk replacer powder to the same amount of water is one method dairy producers and calf feeders use to meet this need. But what happens when too much powder is put into the same amount of water?

Milk replacer powder feeding

There are quite a number of different kinds of milk replacers in the marketplace. Knowing what you have and what the mixing requirements are is the first step. However, it is important to evaluate the calves' requirements. This tells you what kinds of daily weight gains you can achieve with the replacer. As an example, we'll look at the feeding programs of three dairy farmers who donated some milk replacer powder and samples of their calf milk mixes.

Farm A was feeding a 22 percent crude protein, 20 percent fat (22-20) standard milk replacer at a rate of 2 quarts two times per day. They were mixing at a rate of 1.25 pounds per gallon, which gave them about 13 percent total solids. Using the National Research Council Requirements for Dairy Cattle for a 100-pound calf, the energy allowable daily gain was about 0.8 pounds per day, and the protein allowable gain was about 0.82 pounds per day in mild temperatures.

Maintenance requirements on Farm A used about 0.81 pounds of the powder. If the temperature was about 32°F, the calf would be using most of their nutrients for maintenance with not much left to gain any weight. If they increased the total dry matter intake to 1.5 pounds of replacer, they would have enough energy for about a half-pound of gain per day with about 15.4 percent total solids in the mix. If they increased the powder to 1.8 pounds per gallon per day, they would have enough energy to grow 1 pound per day, but the mix would be at 18 percent total solids. (In these calculations, we are assuming they are not eating much in the way of calf starter).

Farm B fed a 28-25 milk replacer, 2 quarts two times daily. They mixed at a rate of 1.8 pounds of powder per gallon. At this rate, on paper, a 100-pound calf could gain about 1.7 pounds per day in the summer and about 1.2 pounds in the winter. However, the total solids were about 18 percent.

Continued on page 50

Winter calf feeding checklist

- ☑ Check with your nutritionist or veterinarian on the nutrient requirements for your calves in winter.
- ☑ If adding milk replacer powder to milk, increasing the powder in the same amount of water or putting additives into the milk or milk replacer, check the solids content and the osmolality to make sure you do not exceed 16 percent total solids or 600 mOsm per kg.
- ☑ Increase the total solids in the milk replacer slowly, 1 to 2 percentage points at a time.
- ☑ Do not add electrolyte powders to milk or milk replacers.
- ☑ Provide free-choice water.
- ☑ Add the milk replacer powder before the final volume is reached for the batch.
- ☑ Mix all milk replacers thoroughly to ensure consistency in feeding.
- ☑ Follow water temperatures recommended on the milk replacer mixing instructions.

If a little's good, is more better? cont'd from page 49

Dale Moore
Professor and Director of Veterinary Medicine Extension
Washington State University
damoore@vetmed.wsu.edu

www.progressivedairy.com

Farm C fed hospital milk plus a milk replacer "booster." Their liquid calf feed was more than 18 percent total solids.

> **"Really, anything that is added to the calf liquid feed could result in higher sodium or higher osmolality. Because the calf's blood wants to maintain a specific osmolality, having high osmolality in calf milk replacer or milk could cause diarrhea ..."**

Total solids and osmolality of calf liquid feeds

The measure of total solids includes all of the components in the milk or milk replacer. We can think of it as the total dry matter in the liquid calf feed. The total solids of whole milk ranges from about 12.5 to 14 percent, depending upon the cow breed. Many of the feeding suggestions on milk replacers suggest mixing to 12.5 percent total solids to mimic what calves might see from the cow. The exact limit of percent total solids that can be fed differs among nutritionists – more than 15 or more than 18 percent.

Osmolality is the concentration of solute particles in a solution. The osmolality of whole cows' milk is less than 300 mOsm per kg, the same as it is in calves' blood. For Farm A, feeding their replacer at 13 percent total solids (1.25 pounds per gallon) led to an osmolality of that specific replacer of about 440 mOsm per kg. At 1.5 pounds per gallon (15 percent solids), the osmolality was about 530, and at 1.8 pounds per gallon, the osmolality rose to 660. For Farm B, feeding 1.8 pounds per gallon of the 28-25 replacer resulted in 18 percent total solids and an osmolality of 466. The Farm C solution of milk and replacer had an osmolality of 701.

Although there is somewhat of a relationship between total solids and osmolality, it really depends on what is in the solution. For example, in colostrum samples we have evaluated, we could see 26 percent total solids but an osmolality of only 440 mOsm per kg. Different milk replacers, when mixed at the same concentrations, may yield different osmolalities.

Osmolality and its consequences

Sodium is a major driver of osmolality in fluids. In an outbreak investigation of sick and dying calves due to salt poisoning, researchers at the University of Wisconsin discovered that the use of high-salinity water, adding electrolyte powder to the liquid feed and adding additional milk replacer powder for winter feeding contributed to the outbreak. Really, anything that is added to the calf liquid feed could result in higher sodium or higher osmolality. Because the calf's blood wants to maintain a specific osmolality, having high osmolality in calf milk replacer or milk could cause diarrhea because fluids want to follow the high concentration of solutes in the milk replacer. In this case, that means they come out of the calf's blood and go into the intestine.

Another potential consequence of high-osmolality fluids fed to calves (greater than 600 mOsm per kg) is a delayed abomasal emptying rate. A delay in abomasal emptying could increase a calf's risk for bloat or abomasitis.

Conclusions

It is vitally important to feed additional nutrients to calves in winter to cover their extra body maintenance requirements. However, increasing the amount of milk replacer powder needs to be done carefully to avoid the consequences of ingesting high-osmolality liquid feeds, such as diarrhea, bloat or abomasitis. Different milk replacers may result in different osmolalities, and adding a milk replacer booster to milk could potentially increase the salt concentration and osmolality. Check the consistency of the liquid feeding program and evaluate the total solids and osmolality of those winter calf feeds to make sure the calves get the feed that is "just right." **PD**

Dale Moore is a professor and director of Veterinary Medicine Extension at Washington State University. Helen Floren is a 2nd-year veterinary student at Washington State University. William Sischo is a member of the research faculty in Veterinary Clinical Sciences at Washington State University.

References omitted due to space but are available upon request.

Blood osmolality (serum)

275-295 mOsm/kg

Osmolality = $\frac{\text{Osmoles}}{\text{kg}}$ Mass Based

Osmolarity = $\frac{\text{Osmoles}}{\text{liter}}$ Volume Based

It should not exceed predicted by more than 10 mOsm/kg

That seems right.

Urine should measure 300-900 mOsm/kg
Normal is 500-800

Observation: Guess what?

The reddish layer is forming on top of the purified soluble protein, just like in the original test tube cultures.

The bulk and primary solution is green, & then top layer is turning red. The could be from oxidation. There is a very interesting transformation that is taking place.

Observations:

1. The phosphate treated cultures were highly productive when left to maturity.

The process took approximately 3-4 days to complete. Incubation w/ heating pad (~28°C) is important to the process as compared to unheated cultures.

The process is complete when the solution clarifies and the protein accumulation is complete & sinks fairly quickly to the bottom of the liquid culture. The culture will be an off white culture w/ the phosphate treatment as opposed to the rust color of the non-phosphate treated culture.

The phosphate culture appears to be significantly more productive in protein than the non-phosphate culture but both methods are highly productive.

All of the remaining culture solution can be harvested and filtered for further concentration w/ low heat.

The strained culture medium does indeed test positive for protein (ie polypeptide) w/ the colorimetric reagent test.

The phosphate treated culture, therefore is HIGHLY productive of both the solid protein-filament network and the water soluble protein (polypeptide).

No material from the mature culture is to be discarded as it is all useful. Residual materials in the mature culture after harvesting serves as the seed material for the next culture.

I have also completed an osmometer test on a urine sample. It has come out well within the normal range w/ a reading of 698 mOsm.

Normal range is 500 - 800 mOsm
650 would be mid range, so mildly elevated from mid range.

Leave for scanning.

Now we need to look @ the mature material from the phosphate treated culture under the scope.

Observation:

On the mature phosphate cultures, they have been examined under the scope. 3000x minimum is required to reveal the structure.

All three forms are present
1. Coccus form
2. Protein conglomeration
3. Filament network

In this culture variation, it appears that the protein aggregation-conglomeration dominates the mass of material produced. This is in keeping with the visual appearance of the mature culture. It has a appearance somewhat akin to curdled milk. There is apparently a filament net present but it is not dominant. The filaments appear to be on the order of 2-3 microns in diameter so they are fine threads. They actually appear to be the most productive culture medium in terms of mass produced relative to time. In comparison to the non-phosphate culture, it may produce 3-5 times as much mass in the same period of time.

It is fair to say that it is "slimy" on a slide also. Difficult to produce a thin smear on AFR plate w/out cover slip.

Dec 06 2010

Lots going on in the lab, as usual, as the countdown toward departure near the end of Dec approaches.

1. Observation on phosphate protein during evaporation / concentration

2. Have questions regarding full strength solutions in the diphosphate along w/ 2 different methods of determining molecular weight, both point & slope methods.

3. Ya would also like to test blood serum w/ the osmometer.

4. Very important observation last night with IR comparison between the upstream sample and the phosphate sample. They are the same.

5. How do the IR spectra of the phosphate-protein (soluble) compare w/ the non-phosphate?

6. How about the solid forms of phosphate vs non-phosphate?

7. Can we get a planaria culture active for toxicology studies.

"The Cough" and Colloidal Silver (Anecdotal)

8. Differences in culture production observations:
   1. Heating vs no heating
   2. Solid protein culture seeding effect?

9. Our list:
   1. DNA extraction?
   2. Basic program - osmometer, viroanalyzer?
   3. Taken examination project
   4. Monitor cultures & harvesting
   5. Toxicology project
   6. Index notebooks

Another observation:

There is no doubt whatsoever that colloidal silver (made within) was immediately effective at ending the infamous "chronic cough" situation that was developing again.

The essence of this topic is contained within the paper entitled "Another Marker for Examination (One of Many)" that was recently posted.

I know the progression of the illness well in my body and the paper above explains what is happening and why it is happening, especially with respect to the chronic & persistent cough that develops.

I know the cough well, and I know that colloidal silver has always been the most effective remedy ever for a period of twenty years.

I first had the cough for a 2-3 year period before I was introduced to colloidal silver, sometime near the year 2000.

It is the only direct means there for to eliminate the cough.

I have had the illness episode almost 2 dozen times (at last least) in the last 20 years.

The actual problem goes back as far as NW Montana, however, to 1993-1994 ~ 25 years.

What I have learned, at least in my particular case, is that the bronchial distress has to pursue its course for ~ 2 weeks + nothing can be done or as effective @ reducing it during that period.

After ~ 2 weeks, it is at that time that colloidal silver can become effective.

I do not know why the time delay is required for effectiveness, but it has been so without exception.

In this case, I did nothing w/ colloidal silver for ~ 14 to 16 days. I waited until the chronic pattern set in, especially signified by coughing primarily as a reaction to eating.

This time, in the most dramatic fashion ever seen, the colloidal silver was immediately effective starting w/in the hour.

I have now taken a total of two doses (~1 oz) of colloidal silver w/in approximately 12 hrs and the deep, chronic, persistent cough of the last 14-16 days has immediately abated. It is the most dramatic case I have ever witnessed, similar to when I was given an intravenous antibiotic during a case of blood poisoning in my mid 20's.

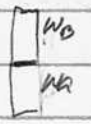
I have never witnessed an inhibitory effect by colloidal silver on a culture (in vitro) thus far.

But I can definitely attest to the benefits of colloidal silver in the body (in vivo)

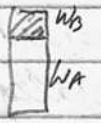
BUT ONLY AT THE RIGHT TIME

It is known now that the "sputum" and the culture are one & the same expression.

Looking @ ratio

1/1 

factor : double $\frac{W_A}{W_B} + 1$

1/2 

factor = 3 $\frac{W_A}{W_B} + 1 = 3$

1/3

1/4 etc

2 WB
3 WA

$\frac{W_A}{W_B} + 1 = \frac{3}{2} + 1 = 2.5$

Ok, some things to square away:

What is the nature of the filaments appearing during evaporation / concentration of the phosphate culture soluble protein?

The microscope, in this case:

Ok, visually, and in creating the slide, everything indicates that it is a denaturing process that is taking place.

The scope is not especially revealing.

I think there is exactly what is going on. It is not a large amount of material but it certainly is enough for further analysis. I would subject it to a pH change and see if it can dissolve.

At this point, we must regard it as a separation process & technique for removing an independent + isolated form of denatured protein.

This could be an important variant of the various protein forms to study.

Moderate heating (~60-65°C) of the soluble phosphate filtered culture medium is denoted by a protein purification method. Denaturing of a solid form by protein (likely similar to the existing conglomerated form, but it may also be unique to some degree since it has been denatured) looks to be very beneficial. Gravity & centrifuging will be used to further separate the purified form.

The soluble, now filtered of the developed denatured protein, is largely a very clear solution now, that has already tested positive for protein w/ the colorimetric test. Currently this is presumed to be of polypeptide form.

—

IR Spectra comparison between sputum sample (Another Marker for Examination - research paper) and the phosphate treated culture - CDB - HEPA based is shown within the next couple of pages. A rather dramatic conclusion, but they are essentially the same thing.

In retrospect, it actually is not a surprise, only dramatic & profound in its implications.

Next:

Let us return to molecular weight determination questions. We now all two options for doing so.

One is a point determination (with conditions attached) and the other is a regression slope determination method. I would like to further sort out the differences between these two.

Lots happening here in the lab so I need to interject these notes as I go along.

The soluble protein is reacting w/ soap. We get a greenish color. The particular soap, however, is known to contain enzymes.

So a question now is, is the protein (polypeptide) reacting with the soap, the enzymes, both, or is it simply a pH reaction?

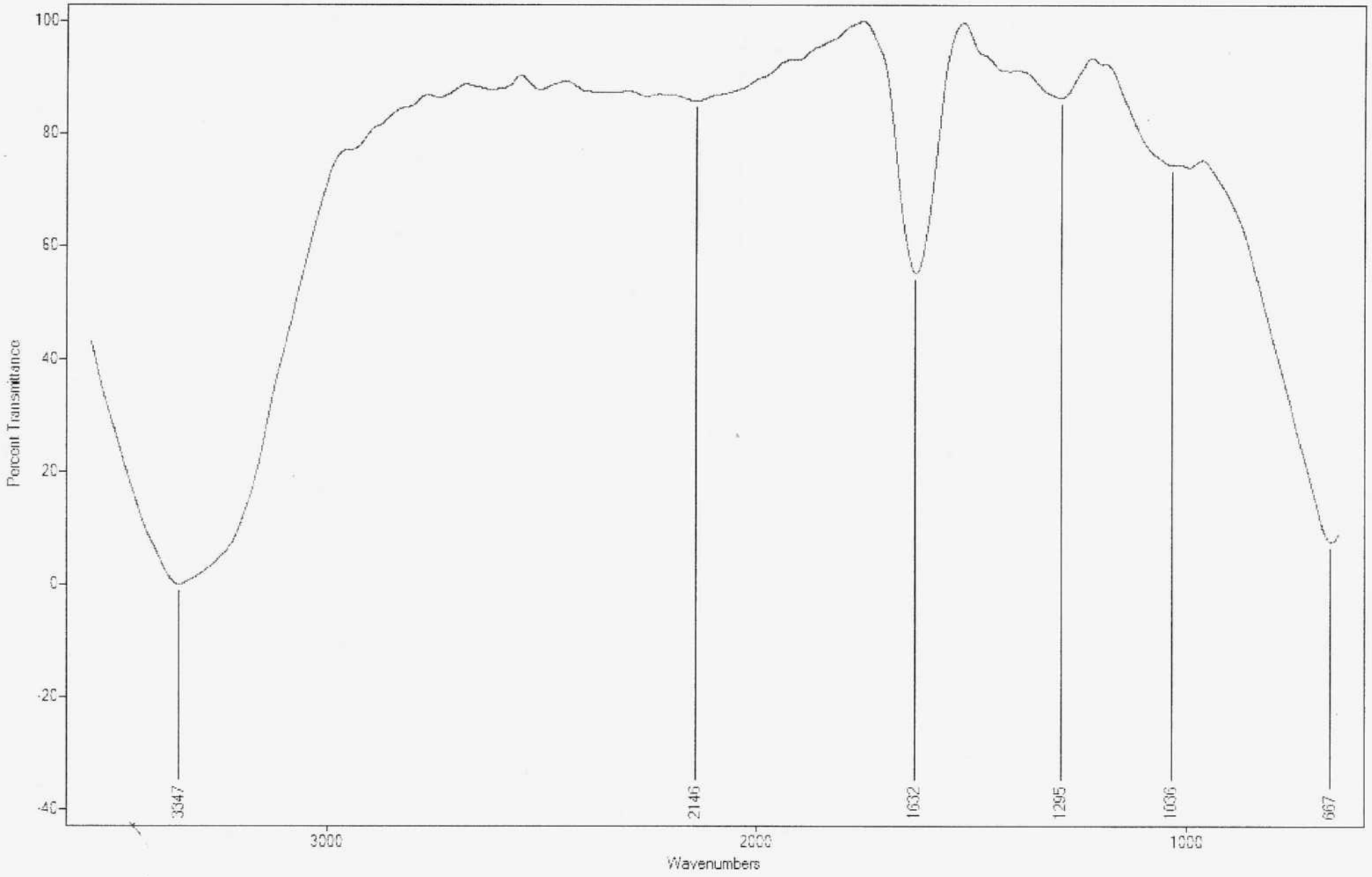
Those are the questions on that observation.

Comparison of Sputum Sample with Phosphate treated HEPA CDB Culture.

Conclusion: They are the same — IR-ATR

CDB Phosphate Protein Culture ATR - Dec 05 2018 - 01.spc: Synthetic spectrum by averaging. See audit trail for details.

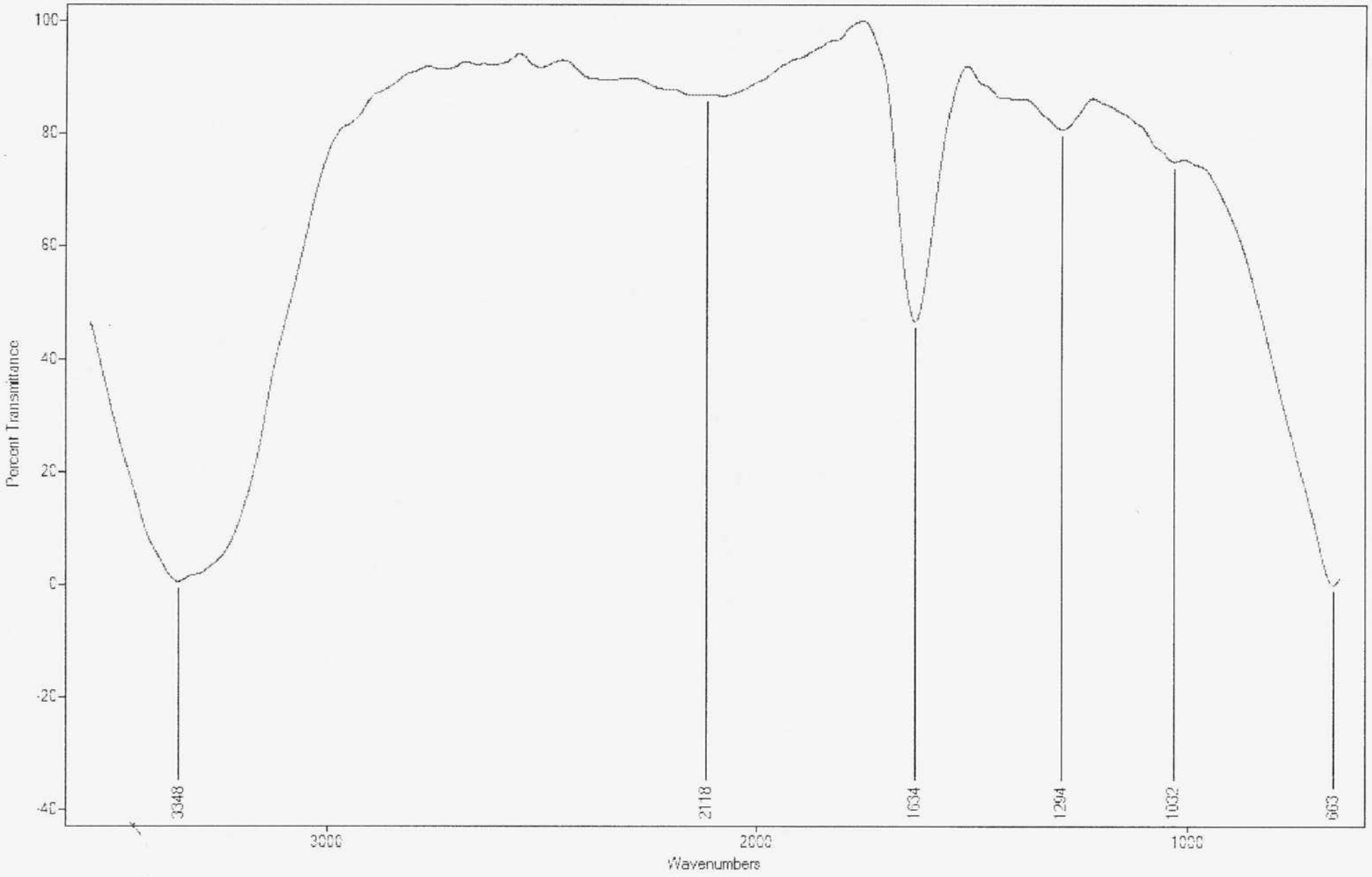
264A



printed on 12/06/2018 03:07:26

Sputum Examination ATR Nov 21 2018 - 01.spc: Synthetic spectrum by averaging. See audit trail for details.

264B



printed on 11/21/2018 15:53:27

Water is removed.

eFTIR

Ok, back to MW methods.

We also learn that full strength solutions,

AS LONG AS IT FALLS WITHIN RANGE OF THE INSTRUMENT,

can be used with osmometer. In fact, there seems to be an advantage in doing so, as this is the convention when it is possible to do so, eg

serum
~~blood~~ urine
milk
] etc.

We also know that we have very good linearity taking place w.r.t. the measurement of dilute solutions.

This means that even if full strength solutions are outside the range of the instrument we can back off the full strength mOsm using the dilute ratio results, as we did with milk.

The question you'd ask is the difference in results between the method and that of the slope of the regression line.

So let's go back to the point where the point method "appeared" to fail, but just maybe it did not fail at all.

We know that we successfully determined the molecular weight of salt using the point method. Those results were extraordinary

The success was achieved on Dec 03 2018 and is recorded in the notes.

We then ran into a problem when we began to look @ milk. First off, there is a difference between a straight forward mOSm reading (such as w/ milk (~300), and urine (~500-800 mOSm) and the determination of molecular weight. That are two totally separate objectives.

So the first is to determine what we want, and I am sure it will usually be both.

We now know milk has an mOSm of ~300 and we have confirmed that result. We can back out the number from dilute solutions as required to do so based upon linearity of the solution.

But we also know that milk has
NUMEROUS PROTEINS

within it, as many things will have
and so there usually will not even be
the means to determine a true
molecular weight for a mixture.

This is even the case for right now with
our soluble protein as we do not know
what type of interference we may be
getting from the culture medium ingredients
themselves.

We can be assured that not all constituents
of the culture medium are entirely used
up in the growth process, so understandably
we already know that we do have a "mixture"
of which a protein (polypeptide) is a
major component. But we do not exactly
know how much that ratio of composition is.

This points out exactly what osmolality is all
about. It is used ~~for~~ exactly for that purpose

To characterize the nature of a complex solution

or, at least, that is one of the major benefits of offers. So you must always keep in mind what exactly are you analyzing, what result are you after, what result can you realistically obtain, and what is the best measurements to use in the process?

The solution showed up perfectly w/ the milk examination. The MOSm on milk was right on target; the protein complexity of milk is a whole different matter.

We determined the "MW" of our milk solution using the slope regression method and ended up @ about 10kda. We know in reality that many proteins are involved of radically different sizes, so for now we are to be cautious in the interpretation of that result.

My question now is, what MW is determined by the point method (subject to restrictions) @ full strength solution? How does it compare to the slope regression method?

I also know that we are going to need to look into the term "effective molar mass" because there is where the issue of mixtures come into play.

So back to our Dec 03 notes, and our proposed equation on the point method:

$$MB = \frac{\left(\frac{WB}{WA}\right) \cdot 1E6 \cdot \text{Van't Hoff factor}}{mOSm}$$

Which we now realize is fundamentally the same as the method identified and developed for the regression-slope method. See our notes of Dec 03 2018 for the work also.

So the problem that came up here is that when WA approaches 0, the solution goes to ∞. And then was the problem — How do you interpret that.

We now see, that because of linearity of the solution, we should have another way to approach the problem when we have a full isotonic solution.

The idea is this:

What is mOSm with milk as the ratio of $\frac{WB}{WA} = 1$?

Ie, a half & half solution?

We see from our notes on Dec 05 2010 that our mOSm measurement is 153.5

From linearity, we know that a full strength solution should have a mOSm/kg of 307 mOSm

Now, my question is, can we solve for a point solution. Yes No, because WB/WA in our equation on the previous page still =1. IS = 1. OK

So now lets use the value of ratio and double that result instead due to linearity:

$$M_B = \frac{\left(\frac{1}{1}\right) \cdot 1EG}{153.5} = 6515 \text{ kDA}$$

And now we double this for full strength solution based upon linearity:

6515 kDA (2) = 13029 kDA

vs our regression box slope result of 10318 DA.

Well, well, well, we certainly are in range and the reasoning is sound. One method is a point solution, the other is a differential method.

Recall that our smallest protein in milk is 14 kDa. Isn't that interesting.

This shows us that we can indeed arrive @ a molecular weight estimate from a single reading, as long as the data is reacted on properly from the dilution ratio - linearity relationship.

Now, notice how salt came out so well. Let's apply the regression method to it.

W_B/W_A	mOSm
.0598	1741
.03	912
.02	611
.012	395

$mOSm = 28363.7\left(W_B/W_A\right) + 52.6 \quad r^2 = .99985$

$\frac{1E6}{28363.7} = 35.3\ (1.8) = \underline{\underline{63.5}}$

vs 58.5

We see therefore, that both methods are actually doing the same thing, and solving the same problem.

It is interesting to me that the point approach actually gave a result closer to the actual value.

3 pt solution: slope = 28423 → MV = 35.2(1.8) = 63.3 gms/mol

The truth is, I am very surprised that there is the much difference between the two methods. One would normally expect the regression method to be superior.

What is the reason for this? Why would the regression solution be larger than any of the point solutions obtained? Why is the point approach more accurate?

What we do know is that:

1. We have two different methods to solve the problem.

2. We want to settle error analysis program in place via BASIC code.

Next we need to investigate our solubility for the protein and what caused us to abandon the point approach.

Well I can see that introduction of the protein molecular wt definitely caused some confusion, and that remains.

I remain very curious about the ~~Correlation~~ of the point approach and the regression slope method.

Let me take a point value and a 3 pt regression on the current protein concentration and compare.

I will use the phosphate protein

FULL	1 mL Protein	0 mL H_2O!
1/1	1 mL Protein	1 mL H_2O
1/2	1 mL Protein	2 mL H_2O
~~1/3~~	~~1 mL Protein~~	~~3 mL H_2O~~

Brix on the current phosphate soluble protein is only 6.9 so we are at the early stages of concentration. Keep that in mind.

1
Wa+1

Page 274

1	[illegible]	[illegible]	0 Full
.5	2	235	1/1
.33	3	133	1/2
	4	134	1/2

Phosphate soluble protein
Brix 6.9
under concentration

mOsm = 182.57 (W_B/W_A) + 46 r^2 = .9983

OK, look @ our salt problem again. W_B/W_A referred to the mass of solute (ie salt) dissolved in the water. That is why this came out so well. In the case of our pistachios WE DO NOT HAVE THIS INFORMATION!

We have no idea what the mass of the protein is that has been dissolved in solution. We do know that the mass is an absolute miniscule fraction of the solution that we have added to the water.

Therefore we can not really calculate what our dilution ratios are. Therefore we can not actually determine the molecular weight. We need to remove all of the water in order to do this. Our dehydrated protein from earlier work is much more well suited to the objective.

Our molecular wgt of the protein is actually probably much higher than we suppose.

Go to the dehydrated protein. This will give you a much better mass dilution ratio.

Add that you can determine now from what you are doing w mOSm, possibly against Brix (index of refraction, etc).

Then you might be able to use your previous model for Brix-index of Refraction — Water Content — Concentration, etc.

This is why w/ milk you are not after a single "molecular weight", you are only after a mOSm value.

We will still look up an "effective molar mass" @ some point.

So the problem is, you are not really dealing with a protein, you are dealing w/ a diluted protein

Weigh boat: 3.27gms
w/ toothpick 3.42gms Δ = .15gms
w/protein added 3.67 Δprotein = .25 gms
w/water added ~~20.55~~ 20.53gms

Covered, no evaporation allowed.
Mass of water: 20.53 - 3.67gms = 16.86gms

Run Complete

Dried Protein

mOsm 58

MULTI

Tech: _____

Date: _____ Sam

ple # mOsm

_____

Calibration

Ok, this gives us an entirely different picture using our highly condensed protein sample from last year. Almost all water should be removed.

$W_B = 0.25$gms
$W_A = 16.86$gms

Point Analysis:

$$m_B \stackrel{?}{=} \frac{\left(\frac{0.25}{16.86}\right)(1000)}{58\ \text{mOsm}} = \frac{256}{\frac{\text{gms}}{\text{mol}}}$$

The point is closer to our original estimate.

Now try a more concentrated solution.

Weightboat 3.37 gms
w/ toothpick 3.48 $\Delta = 0.11$ gms toothpick
w/ water 11.36 $\Delta = 7.88$ water
w/ protein 11.63 $\Delta = 0.27$ gms

$\frac{WB}{WA} = \frac{0.27 \text{ gms}}{7.88 H_2O} = .0343$

1	231
2	133
3	100

Dilution Ratios:
(not really!)

WB/WA	
.0343	Full (actually .0343)
.0172	200ul / 200 ul
.0086	200 ul / 400 ul

Ok, we made it.
We should have 4 good data points now using the fully evaporated protein from a year ago.

~~Try point analyze first~~ !
Try point analyze first.

$\frac{(1/4) 1E6}{133 \text{ mOsm}} =$

$\frac{(.0343)(1E6)}{231 \text{ mOsm}} = 148.5$

$\frac{.25(.0343) 1E6}{100} =$

$\frac{(1/2).0343(1E6)}{133} = 128.9$

85.75

Now look @ regression

(WB/WA)	mOSm	Estimated MW	Error in MW
.0343	231	148	3
.017	133	128	4
.009	100	90	5

$mOSm = 5255.9\,(WB/WA) + 49.0 \quad r^2 = .9951$

$\frac{1}{(5255.9)}\,(IEG) = \underline{\underline{190}}$ gms/mol FOR DILUTE SOLUTIONS ?

Cannot be more than a small polypeptide.

This is an extraordinarily low value.
We need to check the dipeptide idea on the colorimetric test. Dipeptide (Aspartame) fails colorimetric test)
X molecular wgt of amino acid is ~128 Da, not 110 Da as stated in lit sources.

You really need a pure control solution to compare to.
One approach is to project a pure solution you use $\left[\left(\frac{1}{.0343}\right) + 1\right](190\ gms/mol) = 5729\ gms/mol$

This certainly seems more realistic, but how do ya confirm the approach?

We can project the osmolality of a full strength (solution/pure) as

$$\left[\left(\frac{1}{.0343}\right)+1\right]231 = 6965.7\,mOSm$$

Now if a pure protein solution, then would infer a point solution of

$MW \stackrel{?}{=} 143.6$ Assuming Van Hoff factor as 1.?

Albumin (egg white) has a MW of 66 ± 5 kDa

ΔT = no. of Osm $(-1.86^\circ C)$

or no. of Osm $= \frac{\Delta T}{-1.86^\circ C}$ $\quad mOSm = \frac{\Delta T\,(1000)}{-1.86^\circ C}$

also $\Delta t = i\,t f_m$ where m = no. of moles dissolved.

$$mOSm = \frac{\Delta T\,(1000)}{-1.86^\circ C} \quad \text{or} \quad \Delta T = -1.86^\circ C\,\frac{(mOSm)}{1000}$$

Now let's go back to basics w/ salt.

First case: We have W_B/W_A of .0598 w/ MoSo of 1747

Fixed off, we know $\Delta T = -1.86^\circ C\,\frac{(1747)}{1000}$

$= -3.249^\circ C$

I am finding some totally garbage problem answer examples on various net sources.

My work here agrees completely w/ what is a reliable source.

These relations govern the situation:

$-\Delta T = i(-Kf) \cdot \text{molality}$ $\qquad$ $\text{molality} = \frac{\text{no. of moles}}{\text{kg of solvent}}$

* or $\boxed{\Delta T = i(Kf) \cdot \text{molality}}$

Now, we have also established this relationship.

$\Delta T = \frac{Kf \cdot mOSM}{1000}$ but save this for now.

Example: Given: Sample mass = 26.4 gms

Solvent mass = 15 gms

$\Delta T = 5.10^\circ C$ $\qquad$ $Kf = 1.86^\circ C$

Therefore

$$5.10^\circ C = (i)\left(1.86^\circ C \cdot kg \cdot mol^{-1}\right)\left(\frac{\text{no. of moles}}{.015\ kg\ H_2O}\right)$$

no. of moles = .206 moles

Since $\text{moles} = \frac{\text{No of grams of sample}}{\text{MW of sample}}$

$MW = \frac{\text{No of grams of sample}}{\text{no of moles dissolved}} = \frac{26.4 gms}{.206 moles} = \frac{126.2 gms}{mol}$

All ok to here.

OK - Useful Relations

Now lets go back to the NaCl example.

We know that $\Delta T = \frac{K_f \cdot mOSm}{1000}$

We have

$mOSm$ of 1741 so $\Delta T = \frac{(1.86)(1741)}{1000} = -3.25°C$

We also know that

$\Delta T = i(K_f) \cdot molality$ $\qquad molality = \frac{moles}{Kg\ of\ Solvent}$

So $3.25°C = (i)(1.86°C \cdot Kg \cdot mol^{-1}) \cdot \frac{no.\ of\ moles}{.09359\ kg\ H_2O\ (given)}$

So no. of moles $= \frac{0.164}{i}$

moles ~~Mole~~ $= \frac{gms\ of\ sample}{MW\ of\ Compound}$ $\qquad MW\ of\ compound = \frac{gms}{no.\ of\ moles}$

We had 5.60 gms so

$MW = \frac{5.60\ gms}{\left(\frac{0.164\ moles}{i}\right)} = \frac{5.60\ i}{.164\ moles} = 34.2$

And in our case $i = 1.8$ for NaCl $= 61.5$ MW

vs 58.5 actual

OK, good here

OK - Developed Formula and Checked.

Ok, this is fine for salt of the great theme based upon fundamental relationships which are known and now have been checked.

Now let's try to combine our relations and use them.

$\Delta T = i(K_f) \cdot \text{molality}$ $\qquad$ $\text{molality} = \frac{\text{no. of moles}}{\text{kg of solvent}}$

but we also know that $\Delta T = \frac{K_f \cdot mOSm}{1000}$

Therefore

$\frac{K_f \cdot mOSm}{1000} = i(K_f) \cdot \text{molality}$ $\qquad$ K_f cancels

or

$\frac{mOSm}{1000} = (i) \cdot \frac{\text{no. of moles}}{\text{kg of solvent}}$ $\qquad$ or ~~no. of moles =~~

no. of moles dissolved $= \frac{mOSm \cdot \text{kg of solvent}}{1000 \cdot i}$

however, we would like to use gms of solvent

therefore

no. of moles dissolved $= \frac{mOSm \cdot \text{gms of solvent}}{1E6 \cdot i}$

Check this: $\frac{1741}{1E6}\frac{(5.60)}{i} \Rightarrow \frac{93.59}{i}$ $\qquad$ yes, OK

Furthermore:

$MW = \frac{\text{gms of Compound}}{\left(\frac{mOSm \cdot \text{gms of solvent}}{1E6 \cdot i}\right)} = \frac{\text{gms of Compound} \cdot 1E6 \cdot i}{mOSm \cdot \text{gms of solvent}}$

$= \frac{5.60(1E6)\,i}{1741 \cdot 93.59\ \text{gms}} \Rightarrow \frac{\text{gms}}{34.25\,i}$ $\qquad$ $i = 1.06$

$MW = 61.6$

Ok, now we have a functional formula that can be used on a point basis, Good.

$$\text{Molecular Weight} = \frac{(\text{gms of Compound}) \cdot (1E6) \cdot i}{(\text{mOSm} \cdot \text{gms of Solvent})}$$

Now we cannot help but notice that

$$\frac{\text{gms of Compound}}{\text{gms of solvent}} = \frac{W_B}{W_A} \quad !!!$$

Which means we do indeed have thorough validation of the formula

$$\text{Molecular weight} = \left(\frac{W_B}{W_A}\right) \cdot \frac{(1E6) \cdot i}{\text{mOSm}}$$

on a point basis developed from scratch.

We see that this works very well for our salt trial. There obviously has been some difficulties w/ solutions other than salt.

Let's revisit the error analysis -

$$X = \frac{W_B}{W_A}$$

$$Z = \text{mOSm}$$

$$a = 1E6\, i$$

$$y = \frac{aX}{Z}$$

$$\frac{\partial y}{\partial X} = \frac{a}{Z} \qquad \frac{\partial y}{\partial Z} = \frac{-aX}{Z^2}$$

Error Expected in Molecular Weight Determination:

$$(\Delta q)^2 = \left(\frac{a}{z}\right)^2 \Delta x^2 + \left(-axz^{-2}\right)^2 \Delta z^2$$

or

$$(\Delta q)^2 = \left(\frac{1E6\,i}{mOSm}\right)^2 (\Delta^{WB/WA})^2 + \left(\frac{1E6\,i\,(WB/WA)}{mOSm^2}\right)^2 (\Delta mOSm)^2$$

or

$$\Delta q = \left[\left(\frac{1E6\,i}{mOSm}\right)^2 (\Delta^{WB/WA})^2 + \left(\frac{1E6\,i\,(WB/WA)}{mOSm^2}\right)^2 (\Delta mOSm)^2\right]^{1/2}$$

Error in M.W.

Writing a program to solve this will be useful.

OK, we now have the programmed.

OK, this is very helpful. We see that indeed the error does increase significantly as the solution dilution increases. It appears to be somewhat linear, i.e., 4x times dilution we can easily generate 4x the error in the molecular weight determination.

This is very important to know.

Most of the error is coming from error in the weight ratio.

Let's look @ the weight error ratio.

Assume you measure 5.60 gms ± .05 grams
Assume you measure ~~93. gms~~ 93.59 grams +- .05

ratio is $\frac{5.60}{93.59}$ = .060 .0598

now assume $\frac{5.65}{93.54}$ = .0604 this is

an error of .0006 or 0.06%

now assume $\frac{100}{100} = 1$ vs $\frac{100.05}{99.95} = 1.0010$

or .0010

So the error is not likely to be greater than .05%

= 0.1%

Ok, the error is not increasing in respect to the dilution ratio. The error is holding relatively constant. The mass ratio error is a main factor and the number is relatively low as shown above. Your error estimates w/ the salt example are very realistic, on the order of 2-3 gms / mold just as they actually turned out.

Molecular Weight Determination – Comments.

The size of the sample and solvent do not really matter, only the error in the mass ratio is of consequence & that aspect of the error analysis

This is a very good program to have developed.

Now what we see from the error analysis is that the dehydrated protein, and the various proteins in general

are NOT behaving like salt.
I have no idea why, but we do know that the solution is ionic & I suspect there is Complications matter considerably.

Either way, we do seem to be ending up with a very low molecular weight polypeptide, no matter how we go about it, using

1. Point analysis
2. slope regression analysis
3. Molecular weight error analysis

This is where we are @
We have a range of 200-1000 gms/mole in general as our estimate of the MW of the protein.

Let's look into "effective molar mass"

Notice the dehydrated protein sample gives a lower molecular wt estimate, on the order of ~200 gms/mole.

This should be the more pure sample.

The diluted samples give an estimate on the order of ~1000 gms/mole.
These samples should be less pure.

Either way, all indications are that we are dealing w/ a very small protein or polypeptide.

Use of the MW program:

1. Given a certain mass ratio, the higher the mOsm, the lower the molecular weight.
2. Given a certain mass ratio, the error is essentially constant regardless of the mOsm.
3. Given a certain mass ratio, the lower the mOsm, the higher the molecular weight
4. Given a certain mOsm, the lower the mass ratio, the lower the molecular wt and the lower the error in molecular wt.
5. Given a certain mOsm, the higher the mass ratio, the higher the MW and the higher the error in the MW.

6. Given a lower mass ratio with a given mOsm, the MW is lower. Given the above, a decrease in the mOsm will increase the molecular wt.
7. The total error in the MW seems manageable and proportional in all cases reviewed thus far.

---

1. Ok we have the point method computation in place along w/ the error analysis

2. I now also have the regression method in place, tested on the salt example.

I had an error in the decimal place. It all works fine:

NaCl regression is

$$mOsm = 31514.2\left(\frac{WB}{WA}\right) + 16.27 \quad r^2 = .999$$

The inverse of the slope here is 3.17E-5 (I had 31.7E-5) error
When multiplied by the scaling constant of 1E6
we have 3.17E-5 (1E6) = 31.7
And 31.7 (1.8 or 1.9 depending) = 58.3 gms/mol
ie Van Hoff factor

vs actual 58.5 gms/mol

You cannot get any better than this

Ok, I finally see how the Point method is used to advantage. It also uses a regression but uses the intercept vs the slope.

Method is regress Point Molecular Weight = $a\left(\frac{wb}{wa}\right) + b$

The intercept is the molecular weight by the method.

I only find one source, anywhere, for the method and the problem.

The all come from a paper entitled

"Freezing Point of Milk: A Natural Way to Understand Colligative Properties by Mercedes Novo.

It was tricky to sort out all units and derivations within it, but I have it now.

Two differenent methods of regression can be used:

Measured $mOsm = f(wb/wa)$

and

Computed Point Molecular Weight $= f(wb/wa)$ ~~& Molecular Weight Computed from mOsm values~~

Copyright 4 Light

Now we are in a position to go back and review our collected data from the NaCl point onwards!

Guess what, the version of the paper I had did not have the graphs on it, which would have changed everything! It would have made the process a whole lot easier to understand and interpret. But I have it now.

—

We are now in position to interpret our data more properly.

Our first single point analysis of the dilute protein on Dec 03 note gives a MW estimate of 338 gms/mole w/ an error estimate of 5 gms/mole (based upon program developed

We understand we are dealing w/ an "effective molar mass" with our methods.

—

Next we had a 4 pt analysis of the protein sample w/ a Brix of 30 (fairly concentrated)

Points	MW estimates are	Δ	W_B/W_A
	1,599	$\Delta=1$ gms/mole	1/2
	2,533	$\Delta=6$	1/3
Point regression	3,417	$\Delta=4$	1/6
Intercept on this data	4,347	$\Delta=4$	1/9

Is: MW = 298 gms/mole

$r^2 = .962$

The Original Milk Paper - Instructor Notes

291 A

Freezing point of milk: a natural way to understand colligative properties

Mercedes Novo, Belén Reija, and Wajih Al-Soufi

Lab Documentation

Instructor notes

Types of milk

There are two main methods of treatment of raw milk in order to make it suitable for the market, pasteurization and sterilization. While the pasteurization conditions (63-65ºC for at least 30 min or 72-75ºC for at least 15 s) effectively eliminate potential pathogenic microorganisms, it is not sufficient to inactivate thermoresistant spores in milk. The term sterilization refers to the complete elimination of all microorganisms, but the food industry uses the more realistic term "commercial sterilization": a product is not free of all microorganisms, but those that survive the sterilization process are unlikely to grow during storage and to cause product spoilage. Milk can be made commercially sterile by subjecting it to temperatures in excess of 100ºC for a very short time, and packaging it in air-tight containers. The basis of the UHT (Ultra Heat Treated) process is the sterilization of food before packaging, then filling into pre-sterilized containers in a sterile atmosphere. The use of temperatures exceeding 135ºC for 2-5 s enables a continuous flow sterilization process of milk.

The whole milk used in this experiment was pasteurized milk, but UHT whole milk can be used with analogous results. However, it must be taken into account that lactic fermentation of this kind of milk can take much longer than that of pasteurized milk due to the lack of microorganisms. Therefore, the effect of fermentation can be better observed with pasteurized milk. In the case of skim milk, only UHT milk can be found in the Spanish market.

Freezing point of milk

The freezing point is a quite constant property of milk which is usually used to check adulteration by addition of water. Depending on the region of origin the freezing point of cow milk can vary slightly. Also it must be taken into account that the value of the freezing point of milk is affected by a number of factors, such as measuring conditions *(1)*, handling and processing treatment *(2)*, and salt content *(3)*. Nevertheless, the influence of these factors is negligible within the scope of this experiment.

The contribution of the milk constituents to the freezing point was analyzed in the literature *(3)*. It was concluded that lactose, chloride, citrate and lactic acid account for between 79% and 86% of the total freezing point depression. The rest is due to the other components present in milk in smaller amounts, such as phosphates, sodium, potassium, etc. Therefore, the freezing point of a milk sample will mainly depend on its salt content.

We did not find a value for the legal standard of the freezing point of milk valid for the European Union, but only a reference method for measuring it (ISO 5764: Milk determination of freezing point-thermistor cryoscope method). Spanish law advises to use local standards when available. Therefore we have used the legal standard for cow milk coming from our region (Galicia, Spain). In order to perform this laboratory experiment with milk of a different origin, the corresponding legal standard must be obtained.

The freezing point of milk is not affected by the treatment used to eliminate pathogenic microorganisms (pasteurization or UHT process), as shown in the literature *(1)*. Moreover, whole milk and skim milk have the same freezing point since the fat particles do not contribute to freezing point depression, but only those components which are really dissolved. This has been shown in an extensive study with milk samples of different fat contents and very precise freezing point measurements *(1)*.

Effective molar mass of milk

As derived in the next section, Lab Documentation for students, the molar mass determined from freezing point depression values of mixtures is a number-average molar mass where the total weight of solutes is divided by the number of moles of osmotically active particles. Milk contains fat particles and colloidal proteins in suspension which do not contribute to the freezing point depression (so that they counted in the number of moles) but are part of the total weight of powdered milk. Therefore, the mean molar mass obtained for milk is called effective (or apparent) molar mass. Since whole milk contains a much higher amount of fat particles in suspension than skim milk, its effective molar mass is significantly larger. For different samples of skim milk the effective molar mass can vary slightly depending on the presence of fat rests or other colloidal particles. The effective molar mass of milk serum would coincide with the number-average molar mass of the dissolved particles.

With the samples of powdered milk used in this work we obtained an effective molar mass of whole milk about 21% larger than that of skim milk (Table 2 in Lab Summary). The presence of fat in powdered whole milk is about 26% in weight whereas powdered skim milk contains about 1%. This means that in one gram of powdered milk, 0.26 g are not contributing to freezing point depression in the case of whole milk instead of 0.01 g in the case of skim milk. Therefore a difference of about 25% would be expected between the effective molar masses of the two types of milk due to fat content, slightly higher than that we obtained. This can be due to differences

in the contents of colloidal particles in the two types of milk, which also do not contribute to freezing point depression. Skim milk is usually obtained by centrifugation so that colloidal particles may be removed together with fat.

Knowledge on molar masses of food systems is useful for the theoretical study of the physical properties of foods and to understand how the behavior of foods deviates from the ideal solution laws. The use of freezing point depression to determine the effective molar mass of some liquid foods has been reported in the literature *(4)*. An effective molecular weight of 333-356 g mol^{-1} was obtained for freeze-dried skim milk, which is in good agreement with our value taking into account the possible different compositions of the two milk samples. No values were found in the literature for whole milk.

Possible extensions of the experiment

This experiment has been conceived for students of Food Technology attending a general Physical Chemistry course. Nevertheless, it should also be suitable for students of the same level of Chemistry, Biology and Medical Sciences. The first part of the experiment shows the analytical use of freezing point depression to control the quality of milk regarding adulteration by addition of water and by lactic fermentation. Since no quantitative data analysis is needed in this part, it could be also suitable for General Chemistry courses. The second part illustrates the physicochemical use of freezing point depression to determine the molecular mass of a solute. In the case of milk an effective molar mass is obtained which is defined by the composition of the sample, constituting a didactic example to discuss the colligative nature of freezing point depression. The fact that fat and colloidal particles do not contribute to freezing point depression can be also explained on the basis of the different effective molar masses of whole and skim milk. It would be interesting that some students perform the experiment with whole milk and other with skim milk, and that they discuss their results afterwards.

Depending on students' interests and degree, and laboratory resources, the experiment could be changed or extended as follows:

- Part I of the experiment can be nicely used to practise error analysis, since replicate measurements can be made very quickly, allowing calculation of average, standard deviation and 95% confidence limits. In this way the variations of the freezing point observed for the different milk samples could be discussed on the basis of the calculated confidence intervals, and the precision and sensitivity of the method to detect adulteration can be determined.
- In order to compare the behavior of mixtures with that of single solutes, the determination of molar mass could be applied first to solutions of a single component (lactose, for example). This would help the students to understand the method of molar mass determination without the difficulties added by the use of a mixture.

- For students of Food Sciences, other liquid foods could be used instead of milk, such as coffee, grape juice or tomato juice. Effective molar masses of these products are reported in the literature *(4)*.
- For students dealing with biological or medical sciences, it would be interesting to analyze different body fluids such as blood serum or urine. It would be seen that blood serum has the same freezing point as milk serum, since they are in osmotic equilibrium, whereas urine varies widely in concentration and therefore presents a variable freezing point. These results can be explained on a physiological basis, so that the causes for abnormal serum values could be discussed.

Lab Documentation for students

Colligative properties

Colligative properties are a group of properties of solutions which only depend on the number of solute particles present and not on their identity. The four colligative properties are the freezing point depression, the boiling point elevation, the vapor pressure lowering, and the osmotic pressure, considering in all cases the property of the solution compared to the pure solvent. The colligative properties stem from the reduction of the chemical potential of the liquid solvent as a result of the presence of solute, so that they are directly related. For example, two iso-osmotic or isotonic solutions have the same freezing point.

In this work we deal with the freezing point depression ΔT_f, that is the decrease of the freezing point of a solution (T_f) with respect to that of the pure solvent (T_f^*). The relation between the freezing point depression and the concentration of solute in diluted solutions is given by the following equation:

$$\Delta T_f = T_f - T_f^* = -K_f m_B \qquad (1)$$

where K_f is the cryoscopic constant of the solvent and m_B is the molality of solute, i.e. number of moles of solute per kilogram of solvent. This equation indicates that there is a linear relation between the freezing point depression and the concentration of solute, so that addition of solvent to the solution causes a decrease of solute concentration and therefore a smaller freezing point depression.

In the case that more than one solute are present in the solution, the freezing point depression is proportional to the total concentration of particles in solution:

$$\Delta T_f = T_f - T_f^* = -K_f \sum_i m_i \qquad (2)$$

where m_i is the molality of each dissolved particle. Note that a solute which dissociates gives place to two or more particles in solution, each of them contributing to the total freezing point depression. The size of the particle is unimportant so that a single ion (e.g. sodium) contributes

as much to freezing point depression as a single large protein molecule (e.g. albumin). Moreover, particles which are not really dissolved but are in suspension do not contribute to the freezing point depression or to any other colligative property.

Thus the value of any colligative property is directly related to the total concentration of particles in the solution, usually called "osmotically active particles". This leads to define a new quantity, called osmolality (Osm), that accounts for this concentration. The osmole is the number of moles of a chemical compound that contribute to a solution's osmotic pressure and the osmolality is a measure of the osmoles of solute per kilogram of solvent. In terms of freezing point, the osmolality can be defined as the solute molal concentration that causes a freezing point depression of K_f. In aqueous solutions, $K_f = 1.86$ K kg mol^{-1}, so that a solution 1 Osm causes a freezing point depression of 1.86 K (or 1.86°C) and has a freezing point of -1.86°C, since $T_f^* = 0$ for water. For example, human serum is about 290 mOsm, value which corresponds to a freezing point of -0.539°C.

Osmolality is very useful when dealing with mixtures of solutes, as physiological fluids and other solutions of natural origin. The osmolality of these mixtures tends to be dominated by small molecules which are present in high concentrations. For example in serum, sodium, potassium, chloride, bicarbonate, urea and glucose are the only components present in high enough concentrations to individually affect the osmolality. Together these make up over 95% of total osmolality of serum. Large serum components contribute little to the overall osmolality. For example the molar concentration of albumin, the most abundant serum protein, is only about 0.6 mmol/L.

Measurement of freezing point depression

The conventional experimental method for the measurement of freezing points is quite tedious since it involves the use of a well-controlled cooling bath to achieve solid-liquid equilibrium of the sample, and very accurate temperature measurements *(5,6)*. In laboratory courses, the preferred ice-water cooling baths requires the use of organic solvents whose freezing points are some degrees higher than that of water, and usually aromatic compounds as unidentified solutes.

In this experiment we propose the use of a Fiske Osmometer for the measurement of freezing point. This instrument allows an easy and fast measurement of freezing point depression of aqueous solutions. It is extensively used in clinics for determination of body fluids osmolality, thus providing clinical information not available by any other means. A great advantage of Fiske Osmometers is their speed, since a typical measurement takes only about 90 seconds. Moreover, usually very small amounts of sample are needed, in the range from 1-3 milliliter to a few microliter (15-20 µl).

In a Fiske Osmometer, the sample is cooled several degrees below its freezing point (Figure 1). The supercooled sample is then violently agitated so that rapid crystallization takes place. During the freezing process the released heat of fusion causes the temperature to rise up just to the solution's freezing point, that is, the solid-liquid equilibrium temperature.

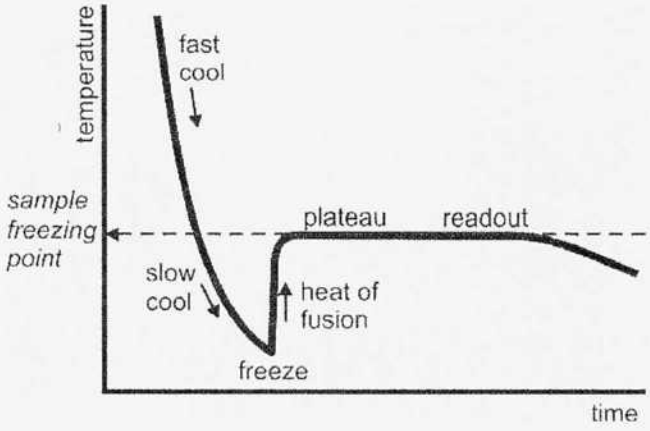


Figure 1. Typical test cycle in a Fiske Osmometer.

To get correct measurements with a Fiske Osmometer, the samples must be well homogenized. Much care must be taken to avoid formation of bubbles, since they cause false readings.

Molecular mass determination

A typical application of colligative properties is the determination of the solute molecular mass. The four colligative properties can be used for small-to-medium sized molecules, whereas only osmotic pressure is sensitive enough for large solute molecules of high molecular mass.

In the case of a single solute which does not dissociate, the following relation between the freezing point depression and the solute molecular mass (M_B) can be derived from equation 1:

$$\Delta T_f = -\frac{K_f}{M_B}\frac{w_B}{w_A} \tag{3}$$

where w_B is the weight of solute and w_A is the weight of solvent. Using this relation, the molecular mass of the solute is obtained from the slope of the plot of the freezing point depression against the ratio of weights of solute and solvent. If the freezing point depression is expressed in osmolality, then the slope of the line is the inverse of the molecular mass of the solute.

Equation 3 is only valid for diluted solutions and must be refined with higher order terms for concentrated solutions *(6)*. Therefore, a more accurate determination of the molecular mass can

be achieved by extrapolation to zero solute concentration in the plot of the solute molar mass values calculated for each solution versus the corresponding weight ratios solute-solvent. M_B is obtained from the experimental data using the relation given by equation 3 reordered as follows:

$$M_B = \frac{K_f}{-\Delta T_f}\frac{w_B}{w_A} \tag{4}$$

When the solution contains a mixture of solutes, the same procedure can be used but the resulting molar mass is a mean molar mass that we call $\bar{M}_B$. The relation between $\bar{M}_B$ and the molecular masses of the particles present in the solution (M_i) can be derived from equation 2 as follows:

$$\Delta T_f = -\frac{K_f}{w_A}\sum_i n_i \tag{5}$$

where n_i is the number of moles of solute i. Comparing equation 5 with equation 3, an expression for $\bar{M}_B$ is obtained:

$$\bar{M}_B = \frac{w_B}{\sum_i n_i} = \frac{\sum_i n_i M_i}{\sum_i n_i} \tag{6}$$

where w_B the total weight of solutes. This expression corresponds to a number-average molar mass (as that defined for polymers), where the total weight of solute is divided by the total number of moles of particles, so that a mean molecular mass of the particles present in the solution is obtained.

Effective molar mass

In this experiment we determine the value of $\bar{M}_B$ for milk, using the weight of powdered milk as total weight of solutes (w_B). Milk contains solutes which do not dissolved in water but remain as particles in suspension. These molecules contribute to the total weight of powdered milk but not to the number of dissolved particles, which constitute the denominator in equation 6. Therefore, the mean molar mass obtained for milk is an effective molar mass. When comparing whole milk with skim milk, the number of osmotically active particles is the same but the amount of fat and other colloidal particles differs. Therefore, the effective molar mass of whole milk is larger than that of skim milk. In both cases the effective molar mass obtained is not the number-average molar mass of the dissolved particles, although it is close to it for skim milk. For practical purposes the inverse of the effective molar mass gives the number of osmotically active particles present in 1 kg of powdered milk.

The concept of effective molar mass is useful in Food Technology since it allows one to estimate the molar mass of a complex mixture such as food. This molar mass is closer to the

number-average molar mass of the solutes the less colloidal particles are present. The knowledge of a molar mass facilitates the theoretical study of the physical properties of foods.

Experimental Procedure

Part I: Quality test of milk.

- Prepare the different samples in test tubes: fresh whole milk, whole milk adulterated with 5% water, whole milk adulterated with 10% water, fermented whole milk and skim milk. Use a 10 ml graduated pipette to prepare the samples with added water. Make sure that the samples are well homogenized.
- Measure the osmolality of the samples, doing at least 3 repeats for each sample with different aliquots. Write down your results in the first three columns of the following table:

Sample	Osmolality / mOsm				Mean T_f /°C
	Reading 1	Reading 2	Reading 3	Mean	
Whole milk					
Whole milk + 5% water					
Whole milk + 10% water					
Fermented whole milk					
Skim milk					

- Calculate the mean osmolality and the mean freezing point of each sample. Write down the values in the fourth and fifth columns of the table, respectively.
- Compare the freezing point of whole milk with the legal standard.
- Compare the values obtained for the freezing points of whole milk and skim milk and explain them.
- Compare the freezing points of the two adulterated samples with that of whole milk. Explain the differences on the basis of the amount of water added in each case.
- Compare the values obtained for the freezing points of fermented and fresh whole milk and explain the differences observed.

Part II: Determination of the effective molar mass of milk.

- Prepare 5 samples of different concentrations of milk by dissolving different amounts of powdered milk (in the range between 0.010 and 0.100 g) in the same amount of water (1 ml or 1 g). Write down the weights of powdered milk and water and the ratio between them in the first three columns of a table like that shown below. The precision of the balance should be at least 1 mg. The amount of water can be measured with a pipette or weighted using the

balance. The samples must be shaken for several minutes to facilitate solubilization and homogenization.

Sample	w_B /g	w_A /g	w_B / w_A	Osmolality / mOsm		
				Reading 1	Reading 2	Reading 3
1						
2						
3						
4						
5						

- Measure the osmolality of the samples, doing at least 3 repeats for each sample with different aliquots. Write down your results in the corresponding columns of the table.
- Plot the osmolality values against the ratio of weights solute/solvent for each sample and draw a straight line going through the data.
- Make a linear regression of the data to get the slope, which is the inverse of the effective molar mass as given by equation 3 (note the osmolality is $-\Delta T_f / K_f$). Calculate the effective molar mass of milk with its error.
- Using equation 4, calculate the values of molar mass for each osmolality value and plot them against the ratio of weights solute/solvent. The linear regression of this data gives the intercept, which is the extrapolation to zero concentration of the effective molar mass of milk.
- Compare the values of effective molar mass of milk obtained by the two methods.

Exercises and questions

As a complement to the experiment, the following questions and exercises are proposed to the students:

1. Using the results obtained in Part I for whole milk, calculate the maximal amount of water that can be added to 100 ml of whole milk keeping the freezing point within the legal range.
2. Calculate the amount of powdered skim milk that must be dissolved in 100 ml of water to obtain a milk with the mean freezing point of the legal standard. Compare it with the amount given in the recipe: 1 tablespoonful (≈ 10 g) in 100 ml water.
3. Compare the effective molar mass obtained for whole milk with that of skim milk and explain the difference on the basis of their compositions.
4. Why is the effective molar mass of skim milk higher than the molecular mass of the heaviest component (lactose, M=342 g/mol)?

291 J

Literature Cited

(1) Rohm, H. *Z. Lebensm. Unters. Forsch.* **1993**, *197*, 558-561.

(2) Puhan, Z. *Alimenta* **1984**, *23*, 82-84.

(3) Mitchell, G. E. *Aust. J. Dairy Technol.* **1989**, *November*, 61-64.

(4) Chen, C. S. *J. Food Sci.* **1986**, *51*, 1537-1553.

(5) Halpern, A. M.; Mcbane, G. C. *Experimental Physical Chemistry: a laboratory textbook*, 3rd ed., W.H. Freeman & Company: New York, 2006.

(6) Garland, C. W.; Nibler, J. W.; Shoemaker, D. P. *Experiments in Physical Chemistry*, 7th ed., McGraw-Hill: New York, 2002.

& Data!
Graphs! after I figured it out painfully so ...

Freezing Point of Milk: A Natural Way To Understand Colligative Properties

W

Mercedes Novo,* Belén Reija, and Wajih Al-Soufi

Departamento de Química Física, Facultade de Ciencias, Universidade de Santiago de Compostela, E-27002 Lugo, Spain; *mnovo@lugo.usc.es

Laboratory exercises using natural systems catch students' interest and can integrate important physicochemical concepts. For example, fluid compartments in humans and most animals are iso-osmotic. The freezing point of blood serum is very nearly the same as that of the cerebro–spinal fluid despite their very different compositions. Therefore, these natural fluids are good examples of colligative properties.

We describe a laboratory exercise dealing with freezing point depression of milk that illustrates application of this colligative property from the analytical and the physicochemical points of view. Since milk is a mixture of solutes in aqueous solution, this experiment helps the students to understand that the contribution of each solute depends only on its concentration and not on its size or mass. Moreover, milk contains suspended fat particles and colloidal proteins that do not contribute to freezing point depression *(1)*. By comparing whole milk and skim milk the students can better understand the differences between dissolved and suspended particles.

The first part of the exercise illustrates a quality test of milk based on freezing point measurements, which is an approved, worldwide method to test for adulteration by water addition. The second part of the experiment determines the effective molar mass of milk, a typical chemical application of the freezing point depression technique.

Measurement of Freezing Point Depression

The conventional method for measuring freezing points is tedious *(2, 3)*. We use instead a Fiske osmometer (Fiske Associates), a widespread, reasonably priced instrument that allows an easy and fast measurement of freezing point depression of aqueous solutions. A typical measurement takes about 90 seconds, and usually small quantities of sample are needed. Fiske osmometers give values of osmolality (Osm), a measure of the total concentration of osmotically-active particles in a solution equal to the sum of the molalities of all dissolved particles. In an aqueous solution 1 Osm causes a freezing point depression of 1.86 °C, so that osmolality values can be directly converted into freezing point values using

$$T_f = T_f^* - \left(1.86 \frac{^\circ\mathrm{C}}{\mathrm{mol\ kg^{-1}}}\right)\left(x\ \mathrm{mol\ kg^{-1}}\right) \qquad (1)$$

where T_f is the freezing point of the solution, T_f^* is the freezing point of the pure solvent (in our case water, so $T_f^* = 0$), and x is the measured osmolality.

Table 1. Values of Osmolality of the Different Milk Samples and the Corresponding Freezing Point Values

Sample	Osmolality/(mmol/kg)			Mean T_f/ °C
	Reading 1	Reading 2	Reading 3	
Whole milk	278	275	277	-0.515
Whole milk + 5% water	262	261	263	0.487
Whole milk + 10% water	251	247	249	-0.463
Fermented whole milk	345	343	343	-0.639
Skim milk	286	284	287	0.531

Experimental Procedure

Part I: Quality Test of Milk

The following samples are prepared and analyzed: (i) whole milk; (ii) whole milk adulterated with the addition of 5% in volume of water; (iii) whole milk adulterated with the addition of 10% in volume of water; (iv) fermented whole milk, obtained by leaving fresh pasteurized milk for at least two days at room temperature to undergo lactic fermentation; and (v) skim milk. The osmolality values of each sample are measured with a Fiske osmometer, repeating typically three readings with different aliquots.

Part II: Determination of the Effective Molar Mass of Milk

Powdered milk is used as substance of unknown molecular weight. Both whole or skim powdered milk can be used, although the latter is easier to dissolve. Solutions are prepared by dissolving different quantities of powdered milk in a certain volume of water. Typical quantities are between 0.010 and 0.100 gram powdered milk per milliliter (gram) water. The solutions are homogenized by shaking or stirring for several minutes. Then, osmolality measurements are performed, repeating at least three readings with different aliquots.

Hazards

There are no significant hazards involved in this experiment. Nevertheless, the students should be advised not to drink from the milk samples owing to the risk of contamination.

Results and Discussion

Part I: Quality Test of Milk

Student results are shown in Table 1. For each sample, the values of osmolality obtained in the three repeats are in good agreement, with a standard deviation of less than 1%. From these values, the freezing points of the samples are calculated.

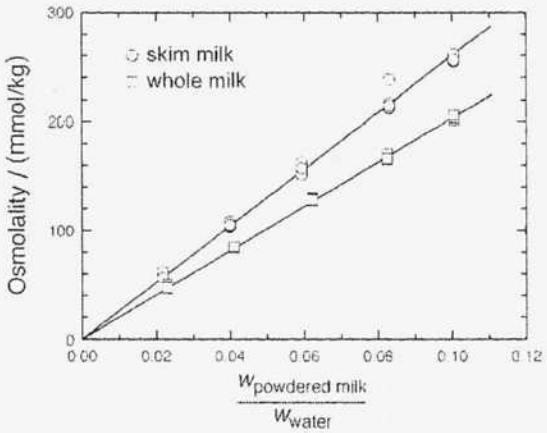


Figure 1. Osmolality values of solutions of different quantities of powdered milk in water. Note that the osmolality values are directly proportional to ΔT_f (eq 2).

The value obtained for the freezing point (T_f) of whole milk is perfectly in agreement with the legal standard given for cow milk from our region (Galicia, Spain): $T_f = -0.526 \pm 0.017$ °C (uncertainty indicated as 3 σ). When comparing with the freezing point of skim milk, slightly different values are obtained but both of them are within the legal range. This result may be surprising at first, but one has to consider that only the milk components in solution contribute to the freezing point depression, so that fat particles do not have any effect on it *(1, 4)*.

The data in Table 1 show the sensitivity of the freezing point depression to adulteration by addition of water, even for small quantities as those used in the experiment. Addition of water causes a decrease of osmolality that is significantly larger than the observed uncertainty in this quantity. Moreover, the decrease in osmolality with respect to untreated milk correlates linearly with the quantity of water added, as expected from the linear relation between freezing point depression and solute molal concentration in diluted solutions

$$\Delta T_f = T_f - T_f^* = -K_f m_B \qquad (2)$$

where K_f is the cryoscopic constant of the solvent (in the case of water $K_f = 1.86$ °C kg mol^{-1}) and m_B is the molality of the solution. Addition of water causes a decrease of solute concentration and leads to a less negative freezing point of the solution.

Finally, the effect of lactic fermentation on the freezing point of milk is dramatic (Table 1). An increase of osmolality is observed of about 15–25%, depending on the fermentation stage of the sample. This means that the freezing point of milk decreases owing to lactic fermentation. This can be easily understood since each molecule of lactose yields four molecules of lactic acid after fermentation, so that the number of particles in solution increases significantly with this process. This is a good example of the "colligative" nature of freezing point depression.

Part II: Determination of the Effective Molar Mass of Milk

The plots of osmolality versus weight ratio between powdered milk and water for solutions of whole and skim milk are shown in Figure 1. Linear variations are observed for both types of milk, as expected from the relation between freezing point depression and weight ratio between solute and solvent derived from eq 2,

$$\Delta T_f = -\frac{K_f}{M_B}\frac{w_B}{w_A} \qquad (3)$$

where M_B is the molar mass of the solute, w_B the weight of solute, and w_A the weight of solvent. Using this relation, the molar mass of the solute is obtained from the slopes of the lines in Figure 1. The values for whole milk and skim milk are given in Table 2.

Table 2. Effective Molar Mass of Whole and Skim Milk Obtained Using Different Methods

Type of Milk	M_B/(kg mol^{-1})	
	From Slopes in Figure 1	Extrapolation to Zero in Figure 2
Whole	0.492 ± 0.001	0.478 ± 0.004
Skim	0.384 ± 0.002	0.376 ± 0.006

NOTE: Uncertainties determined from linear regression and given as one standard deviation.

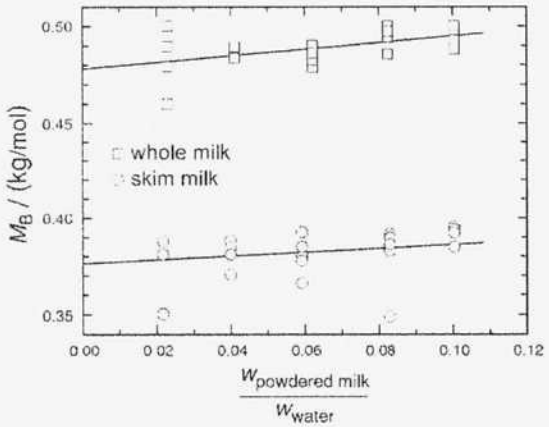


Figure 2. Plot of the effective molar mass calculated for the data in Figure 1.

It is well known that eq 3 is only valid for dilute solutions and must be refined by including higher-order terms for concentrated solutions *(3)*. Therefore, a more accurate determination of the molar mass can be achieved by extrapolation to zero solute concentration in the plot of the solute molar mass values calculated for each solution versus the corresponding weight ratios solute–solvent. M_B is obtained from the experimental data using the relation given by eq 3 reordered as follows:

$$M_B = -\frac{K_f}{\Delta T_f}\frac{w_B}{w_A} \qquad (3)$$

Figure 2 shows the plots of the calculated molar masses versus the weight ratios powdered milk/water corresponding to the data in Figure 1. Variation with solute concentration is small, but a clear decreasing tendency of the molar mass is observed as the milk concentration is decreased. Linear extrapolations lead to the values of molar masses given in

Table 2, which represent the best estimates from the experimental data.

At this point we have to think about the physical meaning of the molar mass obtained for milk. For a solution containing a mixture of solutes, M_B is the number-average molar mass of the solutes (i.e., the total weight of solutes divided by the number of moles of particles in solution). Nevertheless, if particles are present that do not dissolve but remain in suspension, as it is the case of milk, this average molar mass is just an effective molar mass, since these particles have no effect on the freezing point depression but contribute to the total weight of powdered milk. Therefore, the effective molar mass of a mixture depends very much on the presence of suspended particles. This can be seen in the effective molar masses obtained above (Table 2). The effective molar mass of whole milk is significantly larger (about 21%) than that of skim milk. The difference can be explained on the basis of the fat content of the two types of milk, which contributes to the total mass of solute but not to the number of particles in solution.

Acknowledgments

BR thanks the Ministerio de Educación y Ciencia for research scholarship.

[W]Supplemental Material

Instructions for the students and notes for the instructor are available in this issue of *JCE Online*.

Literature Cited

1. Mitchell, G. E. *Aust. J. Dairy Technol.* **1989**, *Nov*, 61–64.
2. Halpern, A. M.; Mebane, G. C. *Experimental Physical Chemistry: A Laboratory Textbook*, 3rd ed.; W. H. Freeman & Company: New York, 2006.
3. Garland, C. W.; Nibler, J. W.; Shoemaker, D. P. *Experiments in Physical Chemistry*, 7th ed.; McGraw-Hill: New York, 2002.
4. Rohm, H. *Z. Lebensm. Unters. Forsch.* **1993**, *197*, 558–561.

Point program to estimate molecular weight and error analysis

```
' Program to estimate Molecular Weight Determinaton Error as well as molecular weight from :


[START]
print
Input "Grams of sample? "; grams
'Print grams
Input "Grams of solvent? "; solvent
'Print solvent
Input "mOsm? "; mosm
'Print mosm
Input "Van Hoff Factor? (1 if unknown) "; vanhoff
'Print vanhoff
unitconstant = 1E6
MW = ((grams / solvent) * unitconstant * vanhoff) / mosm
print
print
print "Point Molecular Weight" + using ("######.##", MW)

'Error Analysis

x = grams / solvent
z = mosm
a = unitconstant * vanhoff

print
xerrorpercent = .05
print "The estimated error in the weight ratio in % is : " + using ("###.##", xerrorpercent
zerror = mosm / 100
print "The estimated error in mOsm is : " + using ("#####.#", zerror)
xerror = xerrorpercent / 100
dysquared = (a / z)^2 * xerror^2 + ((a * x) / z^2) * zerror^2
print
print "Estimate of error in MW" + using ("######.#", sqr(dysquared))

print
print "_____"
Input "Hit any key to continue. "; blank
GOTO [START]
END
```

293B

Grams of sample? .25
Grams of solvent? 1
mOsm? 739
Van Hoff Factor? (1 if unknown) 1

Point Molecular Weight 338.29

The estimated error in the weight ratio in % is : 0.05
The estimated error in mOsm is : 7.4

Estimate of error in MW 5.0

Hit any key to continue.

Regression slope results for the data set:

WB/WA	mOSm
1/2	835
1/3	625
1/6	400
1/9	320

$MoSm = 1323\left(\frac{WB}{WA}\right) + 177.5$

$r^2 = .9995$

$\frac{1}{1323} \cdot 1E6 = 756$ gms/mole

So here we see a large difference (relatively) between the point & slope regression methods

Point data regression			intercept	Slope regression			
338	494	469	(.9995)	756	1050 (.999)	945	
298	419		(.9951)	962	860 (.991)		
462	428		(.999999)	1110	831 (.9899)		

Our next data set is (calc)

WB/WA	mOSm	Point MW	Δ
1/1	1258	794	10
1/2	782	639	1
1/3	572	583	6
1/4	470	532	5

Point intercept MW estimate is 462 gms/mol

$r^2 = .990$

Slope regression estimate is $1E6/1039.6 = 962$ $r^2 = .9959$

—

Next we worked on derivation of equations & unit issues

Our next data set is: CBrNc = 28.5 - fairly concentrated

WB/WA	mOSm	Point MW (calc)	Δ
1/1	1189	841	10
1/2	741	675	1
1/3	561	594	6

Intercept = 494

$r^2 = .999$

Slope: $\frac{1E6}{1110} = 896.9$ ~~884.2~~

$r^2 = .999999$

Our next data set on the problems:

(WB/WA)	MoSm	MW(calc)	Δ
1/1	1229	814	10
1/2	733	682	7
1/3	565	590	6

Point intercept is: 510 $r^2 = .981$

Slope: $\frac{1E6}{956.9} = 1050$ $r^2 = .999$

—

Next set:

WB/WA	MoSm	MW(Calc)	Δ
1/1	1424	702	10
1/2	899	556	7
1/3	644	518	6
1/4	522	479	5

Point intercept: 419 $r^2 = .988$

Slope: $\frac{1E6}{1162.6} = 860$ $r^2 = .991$

—

Next set:

		MW(Calc)	Δ
1/1	1448	691	10
1/2	912	548	7
1/3	639	522	6
1/4	515	485	5

Point intercept: $= 428$ $r^2 = .982$

Slope $= \frac{1E6}{1263.6} = 831$ $r^2 = .9899$

Next:	mOsm	MW(calc)	Δ
1/1	1351	740	10
1/2	807	620	7
1/3	615	542	6

Point intercept: 469 $r^2 = .986$

Slope: $\frac{1E6}{1058.5} = 945$ $r^2 = .9993$

Looking @ the next protein run, we shifted to the highly desiccated protein sample from last year Protein Branches.

Single point analysis gave us MW = 256 gms/mol $\Delta = 9 \frac{gms}{mol}$

We also worked w/ the phosphate protein, highly dilute, Brix = 6.9

WB/WA	mOsm	MW(calc)	Δ
1/1	409	2445	10
1/2	235	2128	7
1/3	133	2506	7

Point intercept: 2329 gms/mole Very interesting! $r^2 = 0$!

Slope: $\frac{1E6}{385.4} = 2590$ $r^2 = .991$

Fascinating — this shows us we may well have a different protein that has formed here.

Notice Consistency between the two methods.

Now the desiccated protein again, from last year

WB/WA	mOsm	MW(calc)	Δ
.0343	231	148.5	3
.0172	133	129	4
.0096	100	86	5

Point intercept: ~~51 $r^2 = .992$~~ 76 $r^2 = .846$

Slope: $\frac{1E6}{5187} = 193$ $r^2 = .992$

Protein
Molecular Wt Data Summary

So now we have:

Dessicated Protein:

Point Regression	Slope Regression
256	193
193 76	

And so these are our data now

(1) Non phosphate treated protein culture
Molecular wt. estimates

Point Regression			Slope Regression		
338	494	469	756	1050	945
298	419		962	860	
462	428		1110	931	

$\bar{X} = 931$ gms/mol $\quad \sigma_{\bar{X}} = \frac{115.3}{\sqrt{7}} = 44$

Modest r^2 values

Note: High r^2 values

Dessicated Protein (from last year):

Point Regression	Slope Regression
256	193
76	

Phosphate Treated Protein

Point Regression	Slope Regression
2329	2590

* Protein Molecular Weight Interpretations. *

We have some interesting interpretation of the data; there are some unexpected results.

1. The high r^2 values of the slope regression method favor those results.

Upon that basis, we propose a molecular weight for the non-phosphate treated culture protein as ~930 gms/mol w/ $\bar{O}_{\bar{x}} = \pm 44$ gms mol.

* If we chose to weight the data, it will shift the estimate closer to ~950 gms/mol w/ $\bar{O}_{\bar{x}} = 35$

The means an expected small protein/polypeptide complex, water soluble, globular, iron-sulfur based.

2. The phosphate treated protein gives unexpected results. It appears to be a different protein variant that is forming here, that it is still a very small protein. MW estimate ~ 2590 gms/mol

3. The desiccated protein from last year has limited value in its interpretation. Unexpectedly the protein in soluble form, diluted is giving the most realistic results.

4. I would like more data on the phosphate protein.

5. The two methods of protein estimation are now understood.

Culture observations..

The phosphate cultures are now doing much better and increasing in production (i.e. the second set) show that they have been alternated to receive the heating pad. I do not have sufficient heating pads to accomodate all cultures.

I have separated and purified the denatured protein that appeared during moderate heating / evaporation of the phosphate soluble protein. It is a solid protein and appears to be especially pure and fine in consistency, more like a powder in solution!

Box of phosphate proteins is now 18.8

Colloidal silver has no visible effects upon the phosphate culture, but it does demonstrate great benefits internally w/ respect the diminishing the "Cough".

Phosphate treated protein.
Moderately condensed/evaporated
Brix @ 18.8

	(W/W)	mOsm	MW(calc)	Δ
1 659	1/1	659	1517	10
2 416	1/2	416	~~1030~~ 1201	7
3 325	1/3	325	1026	6
4 280	1/4	280	893	5

MW: Point Intercept: 746 gms/mol r^2: .969
Slope: $\frac{1E6}{503.1} = 1990$ $r^2 = .9993$

Now we have the following data for the phosphate protein.

~~Point~~	Slope
~~2329~~	2590
	1990
	$\bar{x} = 2290$

Definately also setting in.

There may be an advantage in keeping the protein in a generally dilute stage, i.e, Brix ≤ 10 ??

More data is helpful here

OK we have another set.
Time Correct.

W/W	mOsm	MW(calc)	Δ
~~671~~ 1/1	671	1490	10
~~426~~ 1/2	426	1174	7
1/3	329	1013	6
1/4	280	893	5

Point: MW: 746
Slope: $\frac{1E6}{517.3} = 1933$
$r^2 = .9982$

The phosphate protein MW data is now:

	weight
2590	1
1990	1
1933	3

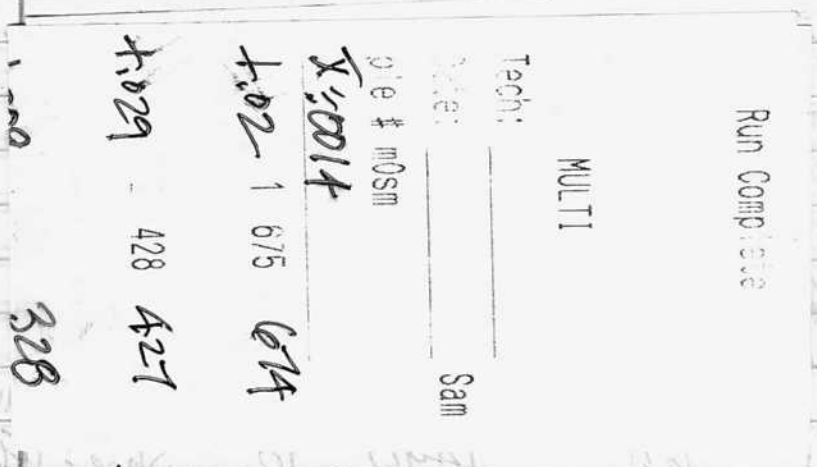
$\sigma = 297.2$

* Weighted mean = 2076 ≒ 2kda

$\sigma_{\bar{x}} = \frac{297.2}{\sqrt{5}} \simeq \pm 133$ (1943 - 2209) ≒ (1950 - 2200)

This is our most valuable data to date on the protein molecular weight current.

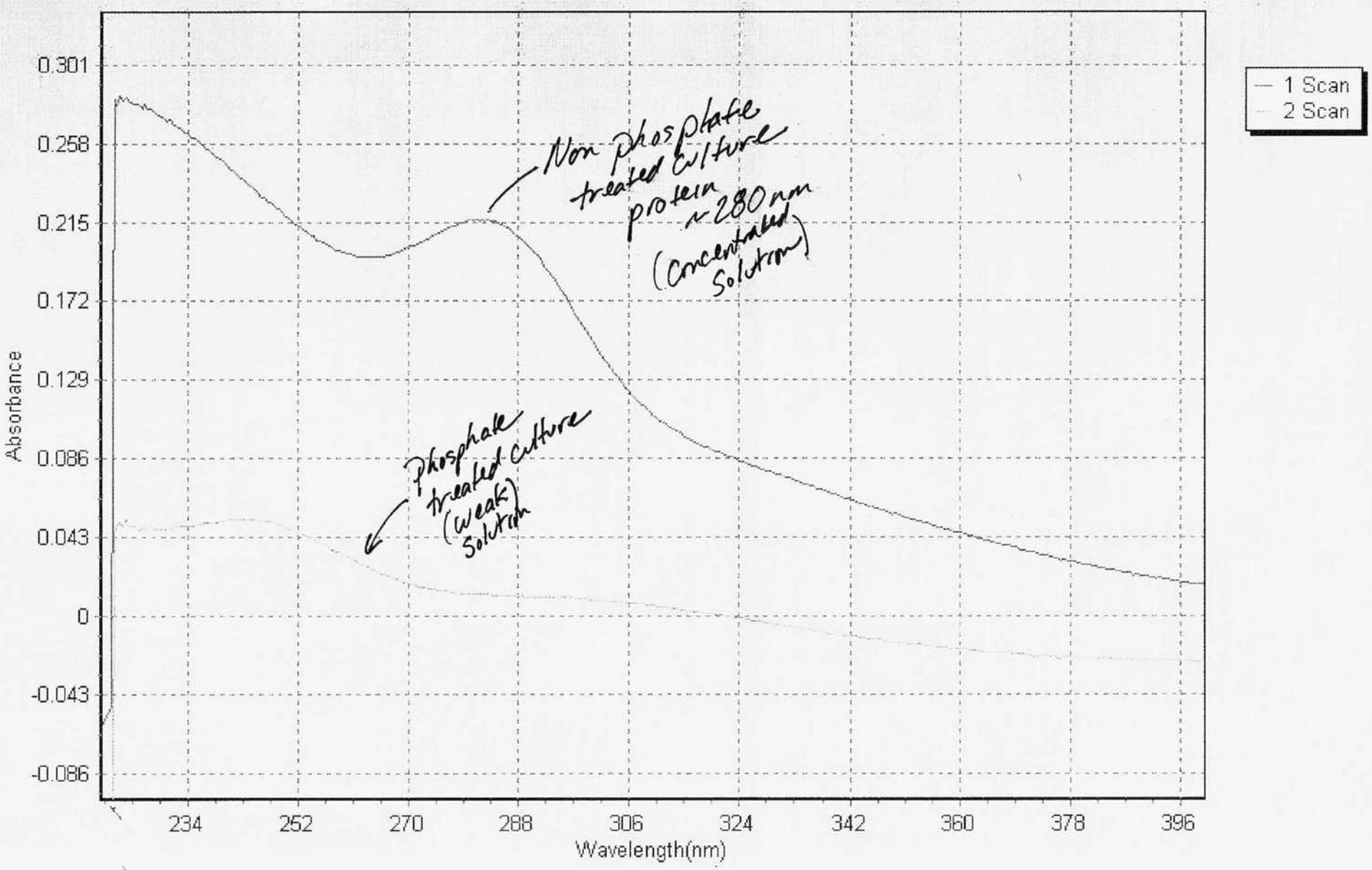
UV & IR analyses is of interest here. Compare the phosphate and non-phosphate proteins.



Leave in place for scan.

Non Phosphate-Phosphate Protein Solutions.jpg

Comparison of water soluble proteins - CDB culture

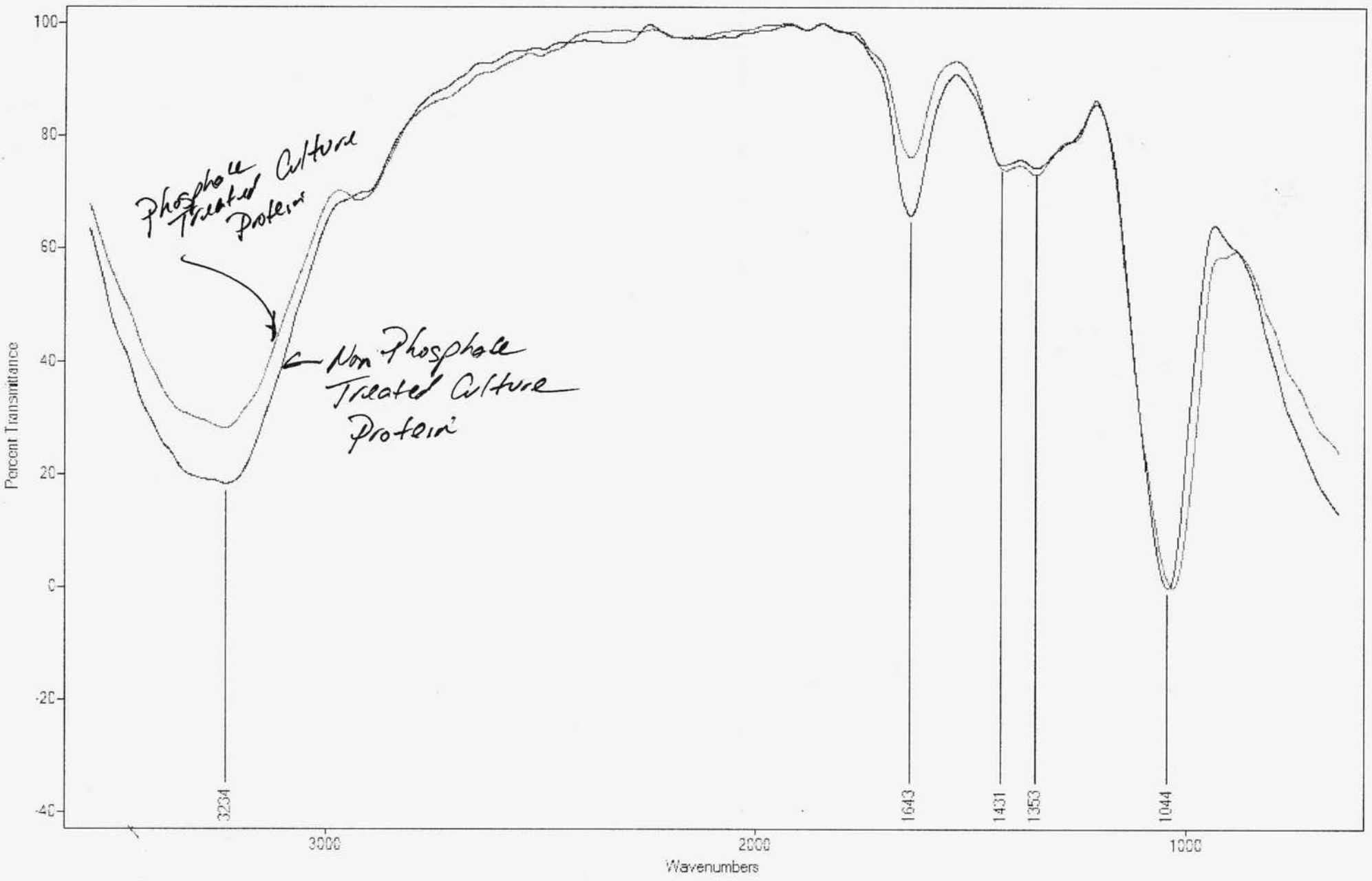


Essentially the same in IR

IR Comparison of Phosphate vs Non Phosphate Protein

CDB Non Phosphate Culture Protein Dec 07 2018 - 01.spc: 12/07/2018 17:47:39 title

303A



printed on 12/07/2018 18:14:22

What we find in comparison between the phosphate and non-phosphate proteases

1. That w.r.t. molality they appear to vary, i.e. also w.r.t molecular weight by a factor of ~2.
2. W.r.t UV they seem to digger, at least at current concentration levels (non-phosphate is considerably more concentrated than the non-phosphate culture.
3. W.r.t. IR they appear to be essentially the same.

Observation:
We now know that each of the soluble proteases (phosphate & non-phosphate) produces a yellow to orange color reaction when mixed with a soap solution.

The particular soap has enzymes in it, so we do not know yet if the reaction is due to the soap, the enzymes, or both.

Dec 08 2018

1. Review the milk data
2. Soap - enzyme - protein question

Milk data is on Dec 05 2018

(w_B/w_A)	mOSM	MW (calc)	Δ
1/1	153.5	6515	10
1/2	106	4717	8
1/3	88.5	3766	8

Point Intercept: MW: 2554 $r^2 = .989$

Slope: $\frac{1E6}{96.92} = 10318\ dA$ $r^2 = .9996$

Again, a major difference between point solution and regression. Regression result will be accepted as best effective molar mass of milk total.

Observation:

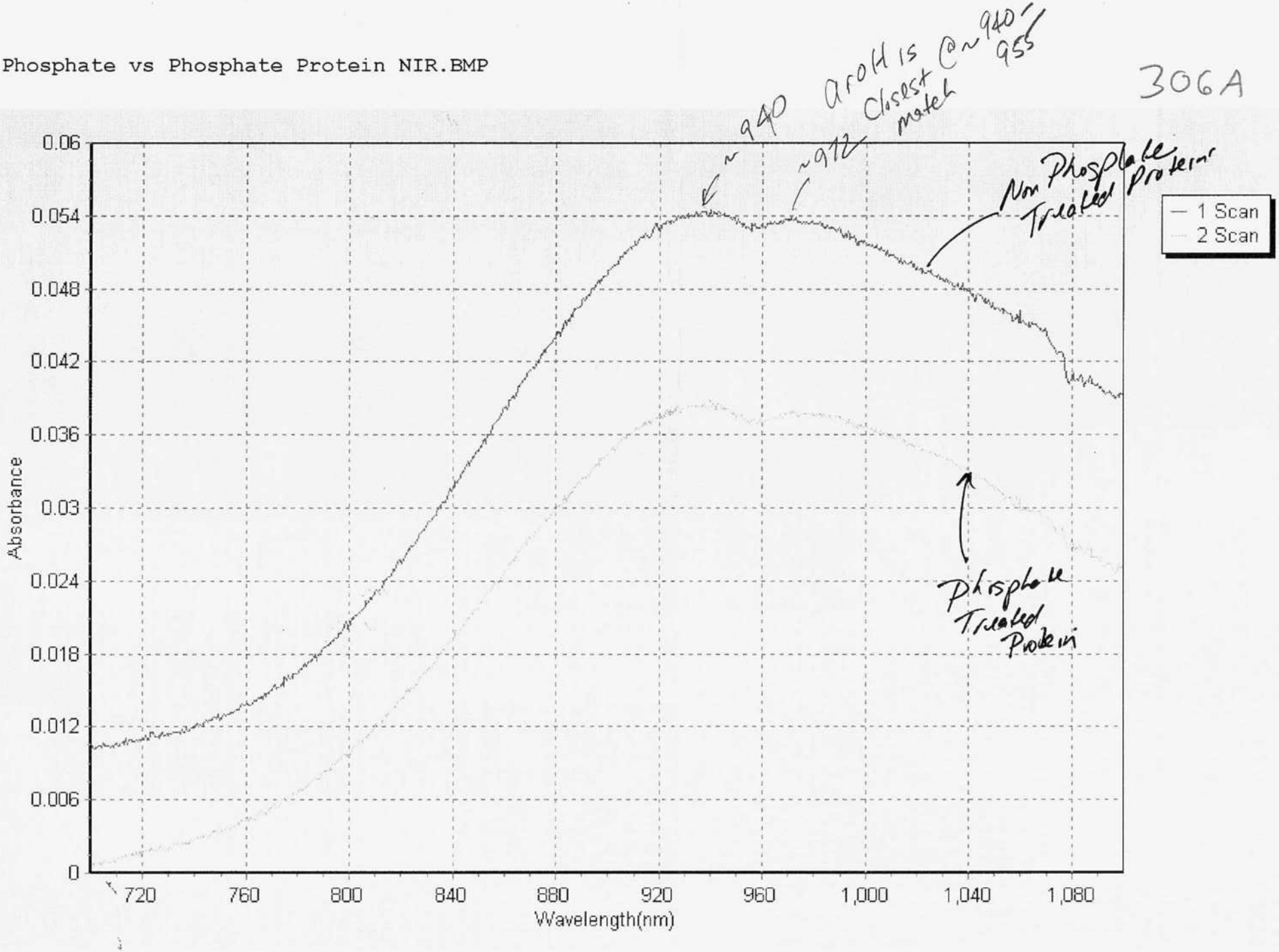
The soluble proteins do appear to be reacting w/ soap, by itself. In alternate, simply, soap has been tested - we still get the color change.

NIR Plot: Phosphate Treated vs
Non Phosphate Soluble Protein
Spectrums

Dec 09 2018

No Phosphate vs Phosphate Protein NIR.BMP

306A



We now have some NIR information on the proteins (both phosphate & non phosphate treated).

We see that both forms of the protein are essentially the same in the NIR region.

We also recall that the IR spectra are essentially identical as well.

We know that we are almost certainly dealing with a polypeptide, and that it is HIGHLY water soluble.

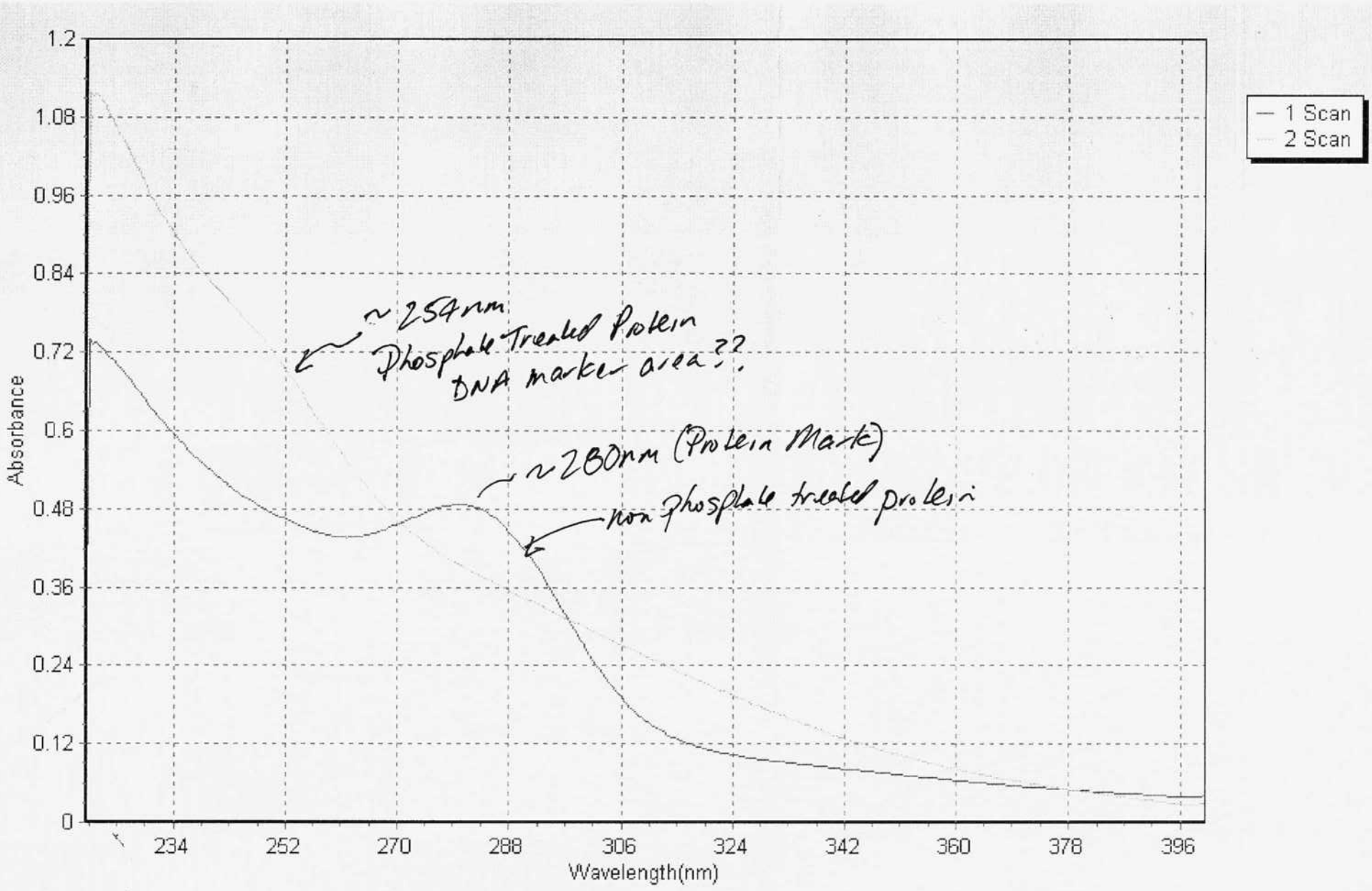
Let's revisit the UV spectrum and try to get the concentrations closer to one another.

What remains of interest here is the difference between the two protein forms in the UV spectrum. Both forms clearly and strongly pass the colormetric reagent test for protein.

NIR & IR show essentially the same spectrum.

But UV gives us a very interesting distinction @ the 280 & 254 nm points — Protein vs DNA?
There is most certainly a question here.

Non Phosphate-Phosphate Protein Solutions.BMP



I think we can again return to the question of what amino acids are likely to be involved in the protein formation.

Now ~~The~~ strong conjecture will be
1. Tyrosine Aromatic OH?
2. Cysteine SH ?
3. Histidine NH? 4. Serine OH
We know we are dealing w/ a strongly hydrophilic protein, ie highly polar.

NH strongly suggests an aromatic hydroxyl is involved, as well as IR seems to fit the same.
OH IR absorption is broad 3300-2500. Overlaps CH. We may well have this.

Tyrosine comes to the fore here.

With the amount of sulfur in the EC culture medium and the strength of the filaments, it seem very difficult to avoid ~~any~~ cysteine in the consideration.

We also know that nitrogen fixation is occurring. The lack of multiple amide peaks in the 3324 absorption area (possibly overlapping w/ OH)

leads me to place histidine strongly on the candidate list.

These are my strongest candidates to begin with thus far.

Serine is also on the list and its biological impact upon the immune system, brain and nervous system deserves its consideration, along with the polar OH group.

Developing tests for these particular amino acids would certainly be helpful.

Examination of the IR absorbance of these amino acids relative to our IR plot of the protein is also a worthwhile endeavor.

Also notice its strong associations w/ Chronic immune suppression diseases.

IR Amino Acid Absorbance

311A

Protein Side Chain Absorptions in the Infrared Finger Print Region

Excerpted from: Lauren DeFlores, *Multi-mode Vibrational Spectroscopy of Peptides and Proteins,* Ch. 6 (PhD Thesis, Massachusetts Institute of Technology, 2008)

See also:

Andreas Barth and Christian Zscherp, "What vibrations tell us about proteins," *Quarterly Reviews of Biophysics* **35** (2002) 369–430

Andreas Barth, "The infrared absorption of amino acid side chains," *Progress in Biophysics & Molecular Biology* **74** (2000) 141–173

Table 1. Extinction coefficients and peak frequencies of amino acids that absorb between 1200 cm^{-1} and 1800 cm^{-1} in H_2O and D_2O. Frequencies are tabulated as a function of pH relative to the pK_a value determined from the isolated amino acid. For vibrations with no pH dependence appear in the central column.

Table 1a. Side Chain Absorptions in H_2O

Amino Acid	cm^{-1} (H_2O) < pK_a low pH		cm^{-1} (H_2O)		cm^{-1} (H_2O) > pK_a high pH		Mode	pK_a
Arginine ARG R	460	1652	-	-	-	-	$\nu_{as}CN_3H_5^+$	11.6 - 12.6
	320	1630	-	-	-	-	$\nu_sCN_3H_5^+$	
Aspartic Acid ASP D	-	-	280	1716			νC=O	4.0 - 4.8
	-	-	-	-	235	1577	$\nu_{as}COO^-$	
	-	-	-	-	256	1402	ν_sCOO^-	
	-	-	-	1375	-	-	δ_sCH_3	
Asparagine ASN N	-	-	320	1677	-	-	νC=O	
	-	-	150	1617	-	-	δNH_2	
Cysteine CYS C	-	-	-	2551	-	-	νSH	9.0-9.5
Glutamic Acid GLU E	-	-	220	1712		-	νC=O	4.4 - 4.6
	-	-	-	-	460	1558	$\nu_{as}COO^-$	
	-	-	-	-	316	1404	ν_sCOO^-	
Glutamine GLN Q	-	-	370	1680	-	-	νC=O	
	-	-	230	1595	-	-	δNH_2	
	-	-	-	1410	-	-	νCN	
Histindine HIS H	250	1631	-	-	-	-	νC=C (H_2')	6.0-7.0
	70	1575, 1594	-	-	-	-	νC=C (H)	
	-	-	-	-	-	1439	δCH_3, νCN ()	
Lysine LYS K	80	1626	-	-	-	-	$\delta_{as}NH_3^+$	10.4-11.1
	85	1526	-	-	-	-	δ_sNH_3'	
Phenylalanine PHE F	-	-	80	1494	-	-	νCC ring	
	-	-	-	1460	-	-	$\delta_{as}CH_3$	
Proline PRO P	-	-	-	1432	-	-	νCN	
	-	-	-	1450	-	-	δCH_2	
Tryptophan TRP W	-	-	-	1622	-	-	νCC, νC=C	
	-	-	-	1509	-	-	νCN, δCH, δNH	
	-	-	-	1496	-	-	νCC, δCH	
	-	-	-	1462	-	-	δCH, νCC, νCN	
	-	-	-	1427	-	-	dNH, νCC, δCH	
Tyrosine TYR Y	120	1617	-	-	-	-	νCC, νCH	9.8 - 10.4
	85	1598	-	-	160	1601	νCC	
	385	1515	-	-	-	-	νCC, δCH	
	-	-	-	-	700	1499	νCC, δCH	
	-	-	-	-	580	1270	νCO, δCC	
	200	1250	-	-	-	-	νCO, δCC	

Table 1b. Side Chain Absorptions in D_2O

Amino Acid	cm^{-1} (D_2O) <pK_a low pH		cm^{-1} (D_2O)		cm^{-1} (D_2O) > pK_a high pH		Mode	pK_a (pH)
Arginine ARG R	460	1605	-	-	-	-	$\nu_{as}CN_3D_5^+$	11.6 - 12.6
	500	1586	-	-	-	-	$\nu_sCN_3D_5$'	
Aspartic Acid ASP D	-	-	290	1713	-	-	νC=O	4.0 - 4.8
	-	-	-	-	820	1584	$\nu_{as}COO^-$	
	-	-	-	-	-	1404	ν_sCOO^-	
Asparagine ASN N	-	-	570	1648	-	-	νC=O	
Cysteine CYS C	-	-	-	1849	-	-	νSD	9.0-9.5
Glutamic Acid GLU E	-	-	280	1706	-	-	νC=O	4.4 - 4.6
	-	-	-	-	830	1567	$\nu_{as}COO^-$	
	-	-	-	-	-	1407	ν_sCOO^-	
Glutamine GLN Q	-	-	550	1640	-	-	νC=O	
	-	-	-	1163	-	-	δND_2	
	-	-	-	1409	-	-	νCN	
Histindine HIS H	35	1600	-	-	-	-	νC=C (D_2^+)	6.0-7.0
	70	1569, 1575	-	-	-	-	νC=C (D)	
	-	-	-	-	-	1439	δCD_3, νCN ()	
Lysine LYS K	-	1200	-	-	-	-	$\delta_{as}ND_3^+$	10.4-11.1
	-	1170	-	-	-	-	$\delta_sND_3^+$	
Tryptophan TRP W	-	-	-	1618	-	-	νCC, νC=C	
	-	-	200	1455	-	-	δCD, νCC, νCN	
	-	-	-	1382	-	-	δND, νCC, δCD	
Tyrosine TYR Y	160	1615	-	-	-	-	νCC, νCD	9.8 - 10.4
	50	1590	-	-	350	1630	νCC	
	500	1515	-	-	-	-	νCC, δCD	
	-	-	-	-	650	1499	νCC, δCD	
	150	1255	-	-	-	-	νCO, δCC	

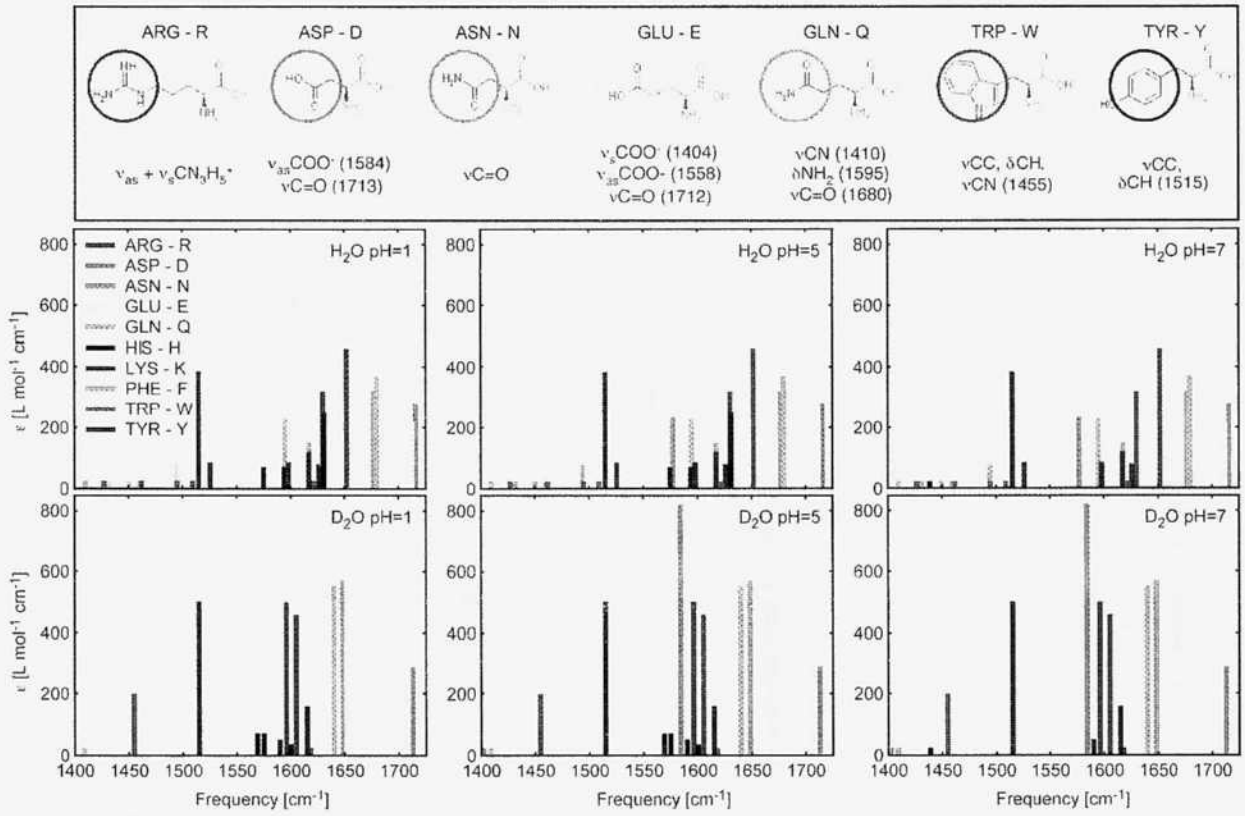


Figure 1. (Top) Atomic structures of predominant side chains in the amide finger region. (Bottom) Stick plots of side chain absorption from Table 1 in the amide finger print region as a function of solvent and pH. Major changes occur due to the protonation state of ASP, GLU and HIS. Isotopic sensitivity of the vibrational absorption is seen in TRP, ARG, GLU and ASP.

312

Serine Amino Acid - Description

312 A

What is serine?

Serine is a non-essential amino acid. It is formed from another amino acid called glycine. Serine is important for both mental and physical health. It has a critical role in ensuring that the central nervous system and the brain are functioning correctly.

Additionally it has a role in forming phospholipids required for cell production. This amino acid is also important in the function of DNA and RNA, muscle formation, and metabolism of fats.

Furthermore, serine is used to produce antibodies. These chemicals are important in supporting a healthy immune system.

Health benefits

Cognition and mental health

The body's nerves are protected in a special layer called a myelin sheath. Serine is crucial in forming and maintaining this sheath. Therefore without an adequate supply of this amino acid, this protective layer can become damaged.

312B

- Cholesterol Reduction
- Diabetes
- Diabetes Type 2
- Hair Loss and Prevention with amino acids
- Immune System
- Inflammation
- Insomnia
- Menopause
- Muscle Growth
- Osteoarthritis
- Rectal Diseases
- Skin and Hair
- Surgery

This reduces its efficiency and it disrupts the signaling between the nerve ending in the body and the brain. This 'short-circuits' mental function and can reduce cognitive ability.

Additionally serine affects the levels of serotonin in the body. Serotonin is an important neurotransmitter that regulates mood. This chemical is produced from an amino acid called tryptophan. Without serine, the body is unable to form tryptophan. Therefore this reduces the amount of serotonin produced. Low levels of tryptophan and serotonin have been linked to insomnia, depression, anxiety, panic attacks and confusion.

Research has also suggested the serine may be beneficial in the treatment of certain mental illnesses such as schizophrenia, [1] [2] [3] [4] [5], Parkinson's disease [6] and depression [7]. These areas continue to be the focus of clinical trials, as well as other diseases including anxiety and dementia.

Fibromyalgia

The syndrome fibromyalgia is a chronic disorder that affects many people. Patients suffer from diffuse tenderness, widespread pain, cognitive disturbance and fatigue. The exact causes of fibromyalgia are unknown, however people suffer

ave lower

3/2C

Recovery

> Virility and Fertility

> Weight Loss

> Well-being

Amino Acids

> Alanine

> Arginine

> Asparagine

> Aspartic acid

> BCAA

> L-cysteine / N-Acetyl-Cysteine / NAC

> Carnosine

> Creatine

> Carnitine

levels of serine in their blood compared with healthy people [8]. Scientists need to conduct more research to establish the role this amino acid may have in this disease. In turn this will determine whether it could help in managing the symptoms of fibromyalgia.

Click to buy serine supplements from Amazon.com

Chronic fatigue syndrome

Myalgic encephalomyelitis, commonly known as chronic fatigue syndrome, is a disease which affects the nervous system. This condition causes muscle pain and inflammation within the spinal cord and brain. Other common symptoms of chronic fatigue syndrome include neurocognitive problems, nausea, weight and blood pressure change, and insomnia. Similar to fibromyalgia, the exact cause of this syndrome is unknown. Low levels of l-serine have been recording in people with chronic fatigue syndrome and this is cause for further investigation[9].

Muscle growth

312 D

- Citrulline
- Cysteine
- GABA (gamma-aminobutyric acid)
- Glutamic acid
- Glutamine
- Glutathione
- Glycine
- Histidine
- HMB
- Isoleucine
- Leucine
- Lysine
- Methionine
- Ornithine
- Phenylalanine

Serine improves the body's ability to absorb another chemical called creatine. Creatine is popular among body builders and other athletes that participate in resistance training. Creatine helps to build muscle mass and supports healthy muscle function, making it also important for cardiovascular function.

Symptoms of serine deficiency

Symptoms of deficiency can include delayed or reduced cognitive and physical skills, seizures, and congenital microcephaly [10]. These deficiencies stem from neurometabolic diseases and defects in the biosynthesis of this important amino acid. In most cases these problems are identified at birth or in young children.

Most people have healthy levels of serine and are able to manufacture sufficient quantities. But during times of illness or other periods of physical stress the production of this amino acid may decline and supplementation may become necessary. As it is not an essential amino acid there are no guidelines for recommended daily intake.

Dietary so

3/2E

> Proline

> Serine

> Theanine

> Threonine

> Tryptophan

> Tyrosine

> Valine

> Taurine

> Whey Protein

To produce serine the body requires sufficient amounts of folic acid and the vitamins B6 and B3. To help boost the body's availability of this important amino acid some foods are particularly important to include in your diet. These include soy-based products, meat, peanuts, and wheat gluten. Unfortunately some people develop allergic reactions to several of these natural sources.

Serine supplements are available in tablet, capsule and powder form. Although available as a stand-alone supplement, this amino acid is more frequently part of combination supplements and sports drinks.

Summary

Serine is a non-essential amino acid. The body manufactures it from important vitamins in the B complex. This amino acid performs a wide range of functions in the body and it is particularly vital for healthy cognitive and immune system function.

Deficiencies are not common, although low levels of this amino acid could lead to health problems.

Maintaining a balanced diet and general good health should help to support adequate serine levels. Dietary supplements are also widely available.

Dec 10 2018

Blood Molality

MULTI

Tech:

Date: Sam

pie # mOsm

317

I have completed successful tests of the molality of blood, urine and by blood.

My urine came in @ ~ 690 mOsm.

Normal range is 50 - 850 so these values seem OK.

My blood molality in serum (quite a trick to obtain) is as shown, 317.

What is the normal range?

275 - 295 mOsm.

295 - 300 is classified as impending dehydration

and 7300 as current hydration

so the test will need to be repeated and it may be that more water is in store. There is also the question of how pure the serum samples are.

Dec 11 2018

I am quite interested in the topic of electrochemical titrations. There is an industry standard and entire instrumentation is devoted to that topic. We have the means to do that w/ the Palmsens.

Titration can be applied to acid-base reactions and redox reactions.

Amperometric or Conductometric seem very well suited to acid-base reactions. I have a conductivity meter however the Palmsens is much broader in its applications and amperometry also seems well suited to the task.

My first trial run (qualitative vs quantitative) w/ KOH as analyte & HCl as titrant seems to be very successful.

Second trial:

Burette @ 15.3 Flow rate = ~~.0094 ml/sec~~ .00667 ml/sec

Read 22 ml @ t = 200 s

24 ml @ t = 2855 still descending. .00533

25 24 ml @ t = 500

26 ml @ t = 625

29 ml @ t = 1250

I am using a Δt interval of 5 sec and a drop rate of ~ 5 sec. Seems like a good pace. The drop rate allows for adequate mixing to take place.

30 ml @ 1700 s Flow rate slowing down w/ less pressure.

Ok, a couple of issues to work through

1. The burette flow rate is not constant. a regression or a pump will be required.

2. You must measure the volume of the analyte very carefully before beginning the procedure.

$ml = 9.9474 t^{.14895}$ $r^2 = .9977$

Predict me there:
(actual 31 ml @ t=

Ok, the flow rate has been reduced essentially to zero. @ Volume = 31 ml. So there is a problem and is why the titration has not reached a discrete movement: You need to make enough solution for several runs and keep the burette full or get a pump.

Ok, I have now worked an air pump into the burette system to see if I can maintain a constant flow rate.

Let's go again.

Chronoamperometry.
Volume of analyte = 20ml

Burette start = 1.3ml

Flow rate determination: 1 drop / 3 secs = better

~~3.0ml @ t=~~
~~3.5 @ t = 265 s~~
~~5 @ t = 515 s~~

12 ml	@ t = 675 s	flow rate
14 ml	t = 765 s	= .022 ml/sec
16 ml	t = 860 s	= ~~.022~~ .0216 ml/sec
18 ml	t = 970 s	= .020 ml/sec
20 ml	t = 1085 s	= .020 ml/sec
23	t = 1315	= .017 ml/sec
26 ml	t = 1605	= .015

Let's change voltage from 0.5 V to 1.0 V =

Pressure is improved but still is decreasing some.

I have improved the air seal

Ok, again

3 ml	@	75 s	
4	@	110 s	= .011 ml/sec
5	@	295	= .009
6	@	410	= .008

I have changed voltage to 1 volt.
I am now getting a cleaner descent.
I also increased concentration of KOH (analyte) by a factor of ~ 2.

Ok, this did not work.

Let's switch to the conductivity meter
Initial volume 2.4 ml EC = ~~3.61~~ 4.20

Ok, now I have set up an automatic titration with the use of a chemel tool (modified).

Ok, this is working well.

2.41 @ 9.3 ml

1 drop titrant every 10 sec.

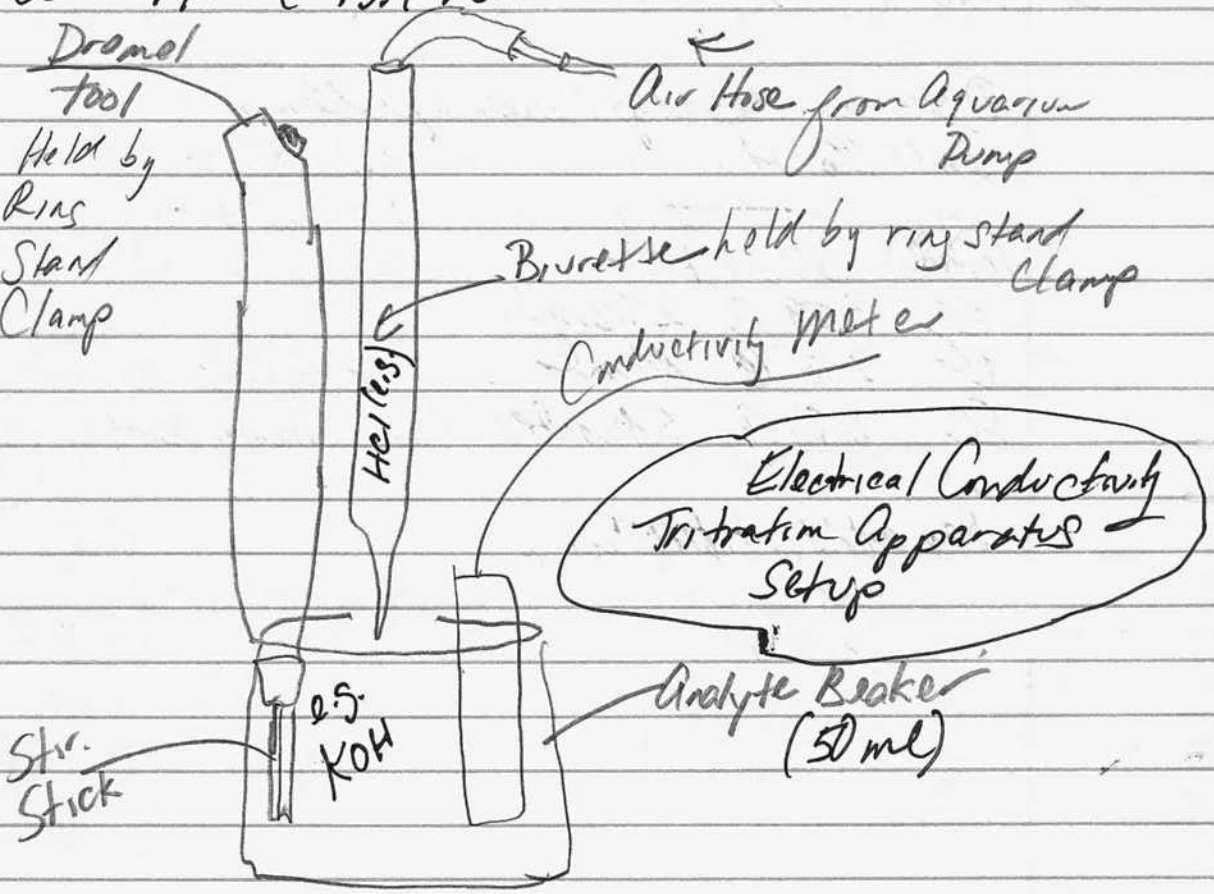
The mixer is a short section of a skewer stick mounted into the small Dremel tool instead of a drill bit.

EC: 1.72 @ 10.0 ml

Having a stirrer is a critical feature that you pay good money for w/ a commercial titrator. $25 Dremel and a clamp stand does the job.

EC 1.36 @ 11.4 ml
EC 1.06 @ 12.0 ml
EC 1.41 @ 12.9 ml
EC 1.31 @ 13.0 ml
EC .90 @ 13.7 ml

use a stool to read value properly.



Amperometry did not produce a smooth.

Ok, the value shot up sharply after reaching a min of 0.90

Stir now stable @ EC 2.41.

Let's repeat.

Initial Conductivity: 23 ml KOH solution

EC 5.05 @ 14.3 ml
EC 2.82 @ 22.1 ml
EC 2.0 @ 25.5 ml
EC 1.18 @ 27.2 ml

Disturbing the setup caused a problem.
Don't do it.

Initial: 30 ml KOH
EC 3.59 @ 27.2 ml
EC 2.15 @ 44 ml
EC 1.16 @ 54 ml

Ran out of titrant.

Again:
15 ml KOH titrant EC 2.30 @ 1.2 ml

I can use either a 50 ml or 200 ml beaker without a problem. The mixer stick must be the correct length however. It can handle up to 2 1/2" skewer stick (just no more) as the mixer.

You can prepare more titrant than you need & then you can process multiple batches if more than 50 ml of titrant is required.

200 ml beaker is preferred as you have lots of room & leeway w/ titrants, analyte, etc. Also less measurement error w/ titrant if you have sufficient sample material. (~15 ml is about the right amount.)

EC 1.90 @ 7.8 ml
EC 1.60 @ 15 ml
EC 1.40 @ 23.0 ml
EC 1.07 @ 31.2
EC 0.92 @ 43.0
EC 0.73 @ 50.0 possible minimum approaching.

Plus

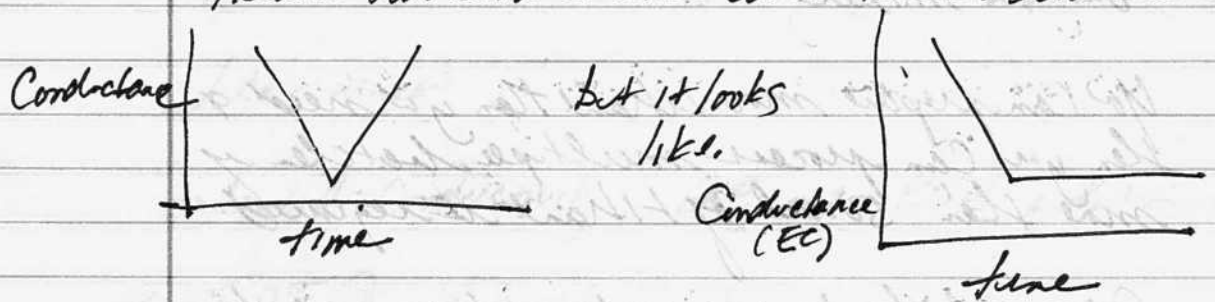
~~0.72 @~~ 0.72 @ 0.2 ml
0.65 @ 2.5 ml
0.66 @ 2.7 ml
0.64 @ 7.0 ml
0.60 @ 10 ml

0.59 @ 12 ml
0.61 @ 14 ml
0.59 @ 17 ml
0.57 @ 20 ml
0.54 @ 24 ml

Dec 12 2018

Titration trials Continued.
We no longer need a pump - Constant delivery rate is not required w/ Conductometric titration

The problem we have is that w/a strong acid-base titration our Conductance curve should look like.



So the team will monitor pH parallel to the Conductance to see more on what is happening.

The first thing that we see is that the EC (Conductance) value is not staying stable. Every time the beaker is disturbed the EC changes. This should not happen since stirring should be equilibrating the analyte.

Ok, we now have a backup Conductivity meter (i.e TDS - PPM). Batteries were dead but fortunately a spare set.

We also have a spare conductance meter in US (<2000).

EC meters only differ by a scaling factor of 1000.
EC_1 = 0.27
EC_2 = 281 (281/1000) = 0.28 pH ≅ 8.5
so we are on the same page. neutral solution.

Lets add KOH analyte to form the analyte.
Range of EC_2 is only ≤ 2000 uS.

Initial Condition then: ~60 ml of analyte.

(uS=X1000) EC_1	uS EC_2	pH	ml (burette)	
2.03	1954	12.2	1.2	Too slow:
1.90	1849	12.2	2.6	Increased HCl in
1.61	1587	12.0	4.2	burette considerably.
1.30	1312	11.5	5.5	
1.2	1220	10.8	6.2	
1.25	1250	9.7	7.0	
1.27	1275	9.3	7.3	
1.28	1295	8.6	7.7	
1.37	1393	6.4	7.9	
1.46	~~52~~1530	5.2	8.2	
1.58	1635	4.2	8.4	
1.70	1760	3.7	8.7	
1.83	1915	3.4	8.9	
1.92	2000	3.3	9.1	
2.1	Max	3.2	9.4	
2.5		3.0	10.0	
3.0		2.9	10.8	
5.0		2.6	14.6	
10.0		2.3	32.5	

Disturbed Setting — End of Data.

Okay, the was a very interesting and good data set.

Several things learned.

1. The 250 ml beaker is very accomodating & easy to work with. Room for 3 meters (2 EC meters, 1 pH) and the stirrer & the burette. 500 ml can be used if needed but it can only accomodate 2 meters.

2. Both EC meters are performing in sync. There is nothing wrong w/ the primary EC meter.

3. Apparently in the trials before our solutions were too weak, ie we apparently did not have a strong acid-strong base titration. I made a very strong titrant this time to increase the span and the titration trial performed flawlessly.

4. There is an interesting lag in the pH data, this suggests the pH meter is not as responsive as the conductivity meter. The points at an apparent weakness in using a pH meter for titration. Error is ~ 2 ml.

5. Let's look @ a regression of EC vs volume.

Looking @ all the data graphically, we definitely have a cusp @ $EC_1 = \underline{1.2}$.

This is the rationale for forming two linear regression and determining their intersect on

It will make sense to cut data to the point of approximately equal conductance on each end.

First data set runs from $E_1 = 2.03$ to 1.2

~~EC = -5.62 (Volume in ml) + 12.98~~ $r^2 = 0.980$

$EC = -0.174(Vol \text{ in } ml) + 2.295$ $r^2 = 0.980$

Second Data set runs from $E_1 = 1.2$ to 2.1
The curve is not linear
It is most definitely parabolic.

$EC = 0.121(Vol)^2 - 1.607(Vol) + 6.54$ $r^2 = .993$

Now the become very interesting because our actual titration point is the intersection of a line and a parabola.

You could use linear work if you restricted the data past the equivalence point.

What we actually have here, however is the situation that:

$y_1 = a_1 x + b$ $\quad\quad$ x = vol in ml
$y = a_2 x^2 + a_3 x + c$
Therefore
$a_1 x + b = a_2 x^2 + a_3 x + c$
$0 = a_2 x^2 + (a_3 - a_1) x + (c - b)$

$a_2 = 0.121$
$(a_3 - a_1) = -1.607 + .174 = -1.433$
$(c - b) = 6.54 - 2.295 = 4.245$

and we have a quadratic equation here. We do not get a real solution for this. Why?

I have looked @ the problem graphically also. There is definitely a real solution.

It occurs @ $x = 5.96$ ml.

—

So I try another calculator and it gives me the following answers:

$x = 5.92 + 0.14i$
$x = 5.92 - 0.14i$

The real portion looks to be correct. Why do we have a complex portion?

Check my math and also

$b^2 - 4ac$

$b^2 - 4ac = (-1.433)^2 - 4(.121)(4.245) = -.001$

Ok, guess what, it is only a case of rounding. The points out the difference between theory and reality.

$b^2 - 4ac = 0$ one real root
> 0 two real roots
< 0 two complex roots.

Obviously the second calculator is much more capable than the first. The problem points out the importance and value of looking @ the "discriminant" : $b^2 - 4ac$ to interpret the expected answer. Obviously we expected ~~or~~ to have at least one real root but only the discriminant revealed the rounding error

1. Which led to the failure of one calculator to even deliver a result, assuming a complex solution had no value.

2. Which led to the proper interpretation of the second calculator result even though it contained a complex portion of a solution.

We know therefore that our approach does make perfect sense and that our solution is @
$X = 5.92$ ml (also graphically as well).

~~We now need the inverse of our regression:~~

$EC = -.174(\text{Vol in ml}) + 2.295$

But not really, we have our solution we could calculate our min EC, however
$EC = -.174(5.92\text{ ml}) + 2.295 = 1.265$
And we read that a C 1.2

However, realizing that a value of 1.265 leads us exactly to a pH = 7.0 VOID VOID

Negative Negative! Wrong Column

This takes care of our lag activation and shows the value of the mutual regression leading to a more accurate result which does in fact coincide

We fully accept 5.92 ml as our most accurate titration point.

It is now that Concentration Determination can proceed. You must keep track of molar ratios when you do this.

$HCl + NaOH = H_2O + Na^+ + Cl^-$ 1 mole each

$M_1V_1 = M_2V_2$ $\quad M_2 = \frac{M_1V_1}{V_2} = \frac{1(60)}{4.72}$

Let M_1 = 1 molar
V_1 = 60 ml $\quad = 12.71\,M$

V_2 = 5.92 ml − 1.2 ml = 4.72 ml
(Origin Point)

The now shows us that the molar concentration of the titrant used is 12.71 times as strong (in terms of molarity) than the analyte.

The matches the titration perfectly, as I once added a bit of concentrated HCl into the burette.

The all appear to be a perfect solution and titration. Your previous results were not quite so smooth w/ weaker solutions.

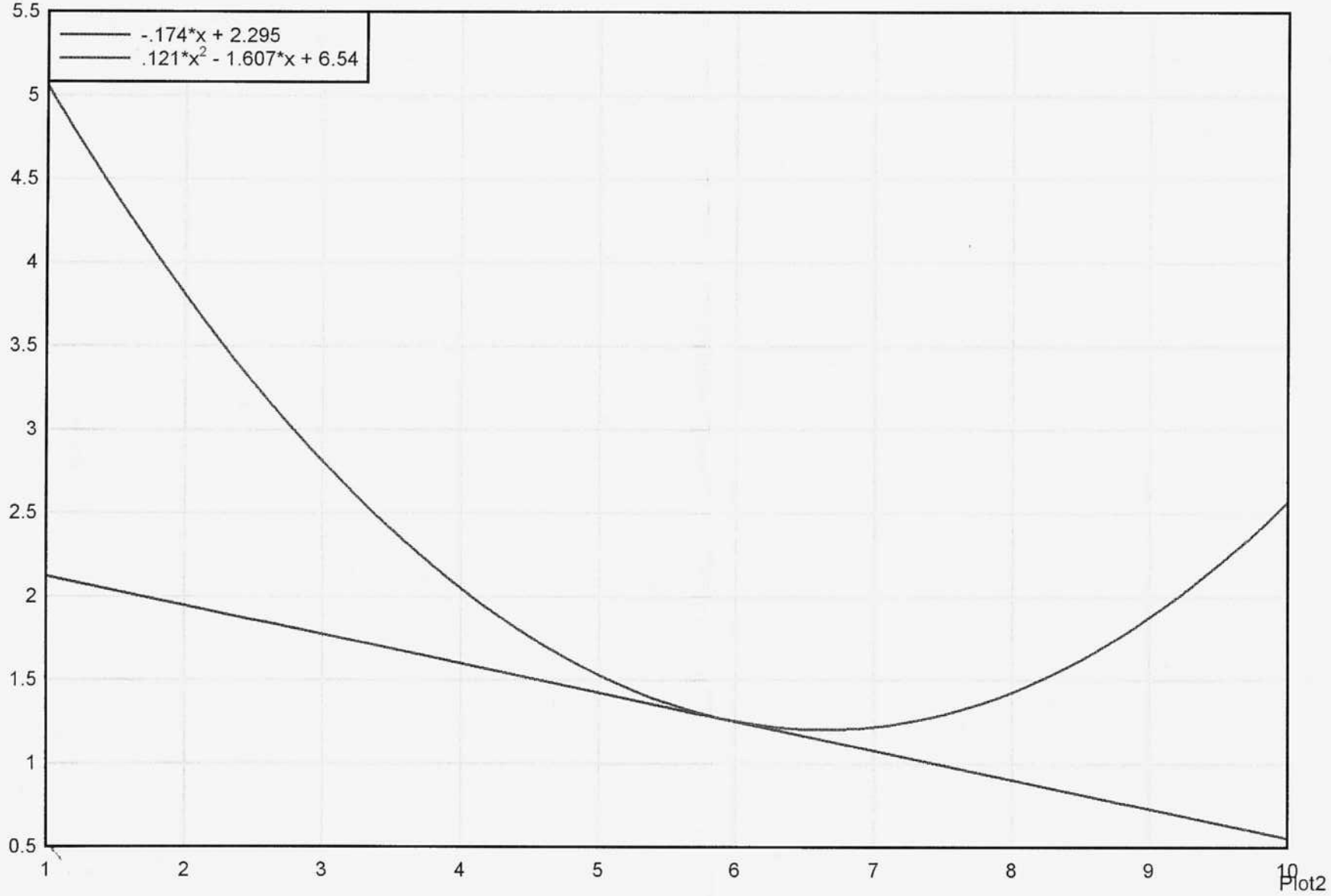
Titrations are indeed an interesting procedure w/ great value — no wonder they are emphasized so much and that Commercial instrumentation has been developed.

That is as far as I can go right now.
The only open the door.

Titration Trial - Graphical solution

Titration: Graphical Solution

329A



Observation on culture:

I have started two trial cultures using ammonium Phosphate, monobasic, as an experiment since this chemical source for phosphates is less expensive.

Result: Within 6 hrs of culture inception, I have the "cellular" units appearing on the surface of the liquid in the culture. I anticipate success with the modification of the culture.

I anticipate that ammonia may also be of greater benefit to the culture than an excess of sodium.

I have now successfully completed a amperometric titration w/ the Pal Scale. I have used pencils as the electrodes, a 250 ml beaker & the dremel mixer. Voltage is 1-2 volt. Current is on the order of 2-5 mA.

Conductance is nothing more than current scaled by the voltage. $US = \frac{I}{V} \cdot 1000$

Very high success again. Approx 1-2 mAmp range using E = 2V, pencil leads, digital measurement of current achieved.

Also record initial volume of analyte.
Your method of recording initial volume, volume of burette & time as round.

It is also reasonable to include a pump to even out flow rate if you have flat luxury.

Palmsens software will determine the regression line w/ ease.

Mathematical solution for t with regression line is relatively easy or we can simply graphically extract from Palmsens.

Attempt to keep the flow rate fairly even & then complete regression of Volume against time.

Subtract residual volume from the predicted regression volume and you have what you need.

$M_1V_1 = M_2V_2$ — only one unknown, i.e. M_2

M_1 can be assumed to be 2.0 for a relative solution.

Dec 13 2018 Page 332

Culture - Protein observation:

The phosphate treated protein has been successfully concentrated and purified. It has the identical green color that the non-phosphate protein had. Recall the primary difference between the spectra was in the UV plots.

Recall that the non-phosphate protein developed a thin red layer on the surface similar to the "red-layer" that tube culture from times past.

The same culture then gradually transitioned to a brownish color over several days to a week.

The phosphate version of the protein appears to be following an identical progression.

1. First, the color of the freshly concentrated protein is identically green in color.
2. The thin red layer is now forming within 1-2 days on the surface.
3. It will now be observed as it transitions to brown. We can only assume this is the result of an iron oxidation process.

Important finding: The mystery of the UV spectral difference between the phosphate & non phosphate cultures of the soluble protein have been resolved.

There is no difference after suitable concentration and purification have taken place. They are the same protein

as revealed in the UV spectra shown on the following page. The former difference has been eliminated when both the protein forms are concentrated and purified at the same levels.

This means that we now have coincidence in UV, NIR & IR regions.

Thus far the primary advantage of the phosphate method of culturing is that it appears to be dramatically more productive of the soluble protein, possibly by a factor of 2 or so. It also produces an off white protein instead of a rust colored protein so it obviously reacts further w/ the iron in the culture medium.

Post Concentration: Phosphate vs Non Phosphate Protein

No Phosphate vs Phosphate Protein UV.BMP

334A