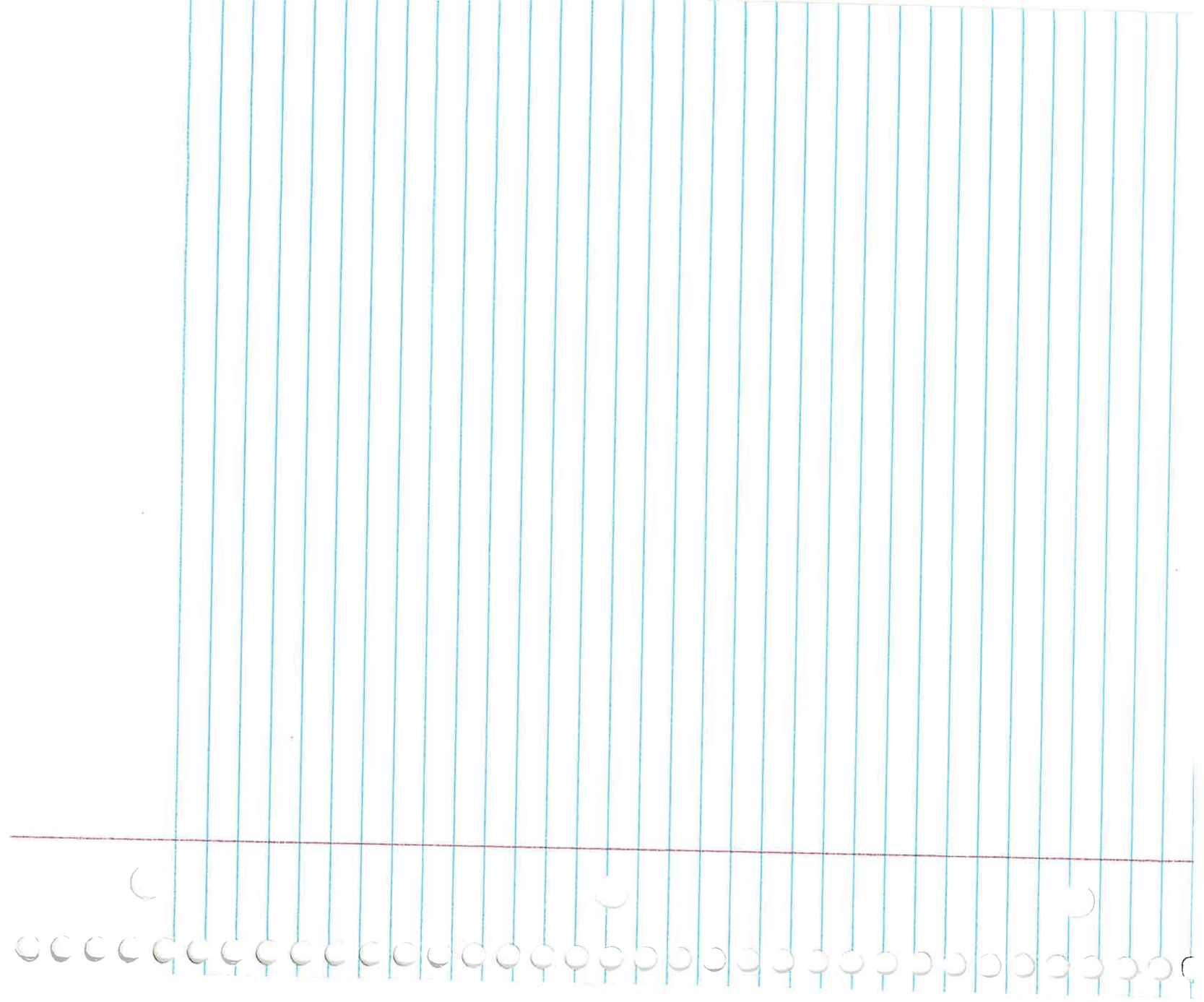
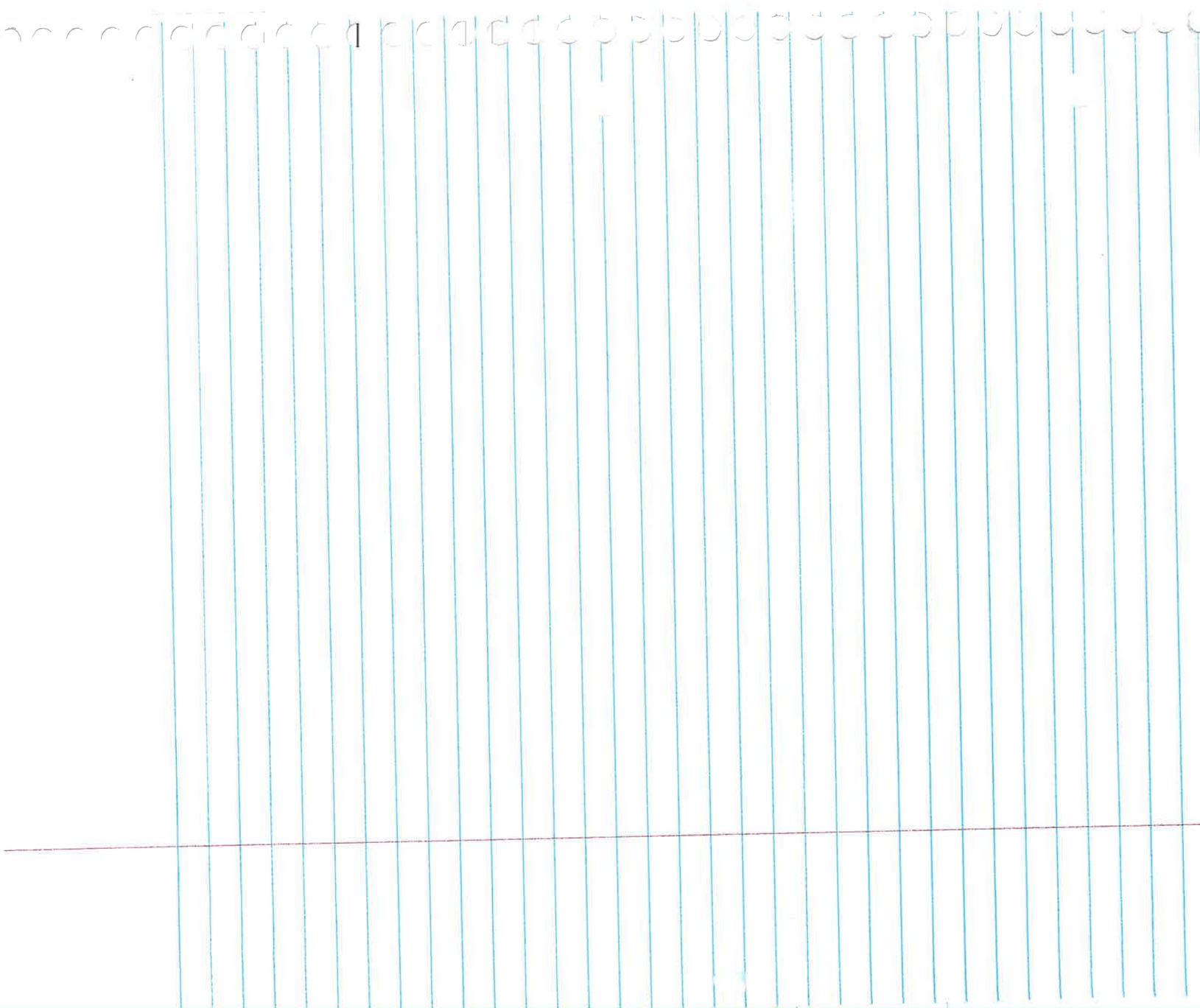
CI LABORATORY NOTEBOOK VOL XXXIII





Oct 08, 2023

There is a good list of strategies and trials ahead:

Trials:

1. Citrate
2. Bicarbonate
3. Vit B, C
4. Enzyme (variable)
5. pH
6. Temperature - Incubation
7. Precipitation or not
8. Buffers
9. Starch, no starch
10. FeSO4, potato
11. Blood culture?
12. Concentration
13. Water urea + beta
14. Magnetics, EMF, AC freq
15. Light blood propagation?

Strategies

1. Buffer development & study
2. Titration in depth acid identification
3. NP determination
4. NIR & electrochemistry
5. Culture development & monitoring is a continuous requirement.

Our two large cultures in development are look very productive. Large culture is agitated @ ~ 114°F and the upper culture is @ ~ 75°F. Not always clear what causes variation in yeast development. Ration are

~ 3500 ml H_2O
4 tbsp soy m
4 tbsp fertilizer
2 tbsp botanek or no starch
1/8 tsp pink salt.
~ 3 days incubation - VOC monitoring (48hr?)

	PPT	
TSP 20 dilution	72	N.S., No Precip 5.7
30	70	N.S., Precip 6.8
40	111	Starch, Precip 6.8

NS No Starch
WS w/Starch

These concentration numbers show the advantage of the large culture volume for subsequent evaporation. Recall that TSP01 had a concentration factor of 3, by collection the should read on a PPT of up to ~240. The PPT meter only goes to ~160 so the explain why we needed to dilute the culture by a factor of 3 just to measure it. We therefore would like to increase our concentration above that in the TSP01 ferment.

Yesterday we looked @ the general TSP 20, 30, 40 series
Lets look @ genl chem version of these (TSP20, 30, & 40)
and inoculations.

Recall that TSP01 required ~2 week for synth blood to
appear, and it was also preceded w/ highly geometric crystals.
Neither of these two events or stages has been replicated but
both were well documented.

TSP40 series may be current favorable prospect for
synth blood development. Both gelling structures have
been recorded.

TSP20 Petri appears null. N.S., PH 5.7, No Precip
TSP20 "Series" (original volume) also appears essentially null.
The culture is returned @ the original pH of ~ 5.7 so
this is in keeping w/ suspected importance of biological
pH being used.

TSP30 Clear activity (Petri) present visually. N.S. Precip, pH 6.8
Asymm. cant Crystal development. Moderate geometry
levels, some internal CDB networking nucleii,
occasional "step facet" (that leads to vernacular development)
on some crystals. This shows that the Continuation of
biological pH and precipitation is important. Protein
development moderate.

TSP "Series" (original volume) shows asymm. cant bottom
layer & light surface layer development. (N.S., Precip, 6.8)

TSP 40 Petri Clear activity present. W/ starch.
Precip, pH 6.8. Visibly darker in color
than TSP 20 or TSP 30 (Petri)

What we have here is:
1. Insignificant protein complex development
2. Insignificant CDB
3. Insignificant CDB small chain development
4. Light crystal development, however internal
organization w/in these crystals may be
higher than that of the TSP 30 "series"
and TSP 30 pellet cultures.

At this point TSP 20 @ acidic pH is considered
a null. TSP 30 appears to favor crystal
development, TSP 40 appears to favor CDB &
protein production.

Neither TSP 30 or TSP 40 is considered to be "more
developed" @ this stage, both are active cultures.
The only major difference known between them
is the inclusion of starch or not.

The is the more specific type of organism in that is
now required. Good. Biological pH beneficial
in both respects.

Concentration, however, is also another important
factor here between TSP 30 & TSP 40.

Now let's look @ inoculations:
1st is TSP40 "series" (original volume) inoculated. pH 6.8
w/ starch. Precip Incubation 60-80°F.

& do see a small surface layer. There was not visible in the petri dish relation. Difficult to capture the layer.

We do have some difference here therefore inoculation may be important. SURFACE LAYER ONLY SO FAR
1. There is crystal development but difficult to capture
2. There are two cases of a transparent filament of uniform geometry where the biological growth within it.
3. There are also fully formed clear filaments appearing, very faint red color
4. COB Chains are a bit longer here.

There is surface layer only. Recall, however, that agate blood layer only just appeared on the surface after ~ 2 weeks. Therefore we have ~ 40 hrs

Now for "bottom layer"
1. Major COB - Major Protein Production layer is on the bottom. No significant crystal development.

The TSP 40 series, esp. inoculated, appear to be a more advanced culture @ this point.

Continue incubation.

TSP 30 "Series" under incubation show significant internal crystal organization pH=6.8
no starch, ~~starch~~, prep.
Both starch and non starch cultures both produce abundant and valuable products.
Neither can be considered "more advanced" than the other.

Continue incubation.

TSP 40 "Series under incubation but pH=7.4
"
1. "Transparent biological tubes" are visible
2. Protein complexes appear denser and more compact.
3. Transparent thin uniform geometry filaments visible
4. No Crystal development of any significance

Continue incubation

I think that we have learned some things from these series:
1. Biological pH, min 6.8 Causes increased activity
2. Crystal development seems to respond well to a non starch culture
3. CDB and min protein Complex formation seems to respond well to a starch culture.
4. Both starch and non starch culture appear to have value; they are just different from one another.
5. Incubation appears to enhance both culture types

6. Increased pH from 6.0 & 7.4 does affect the starch barley culture, suspect the same will be the case for non starch culture as well.

It is TSP11. Sugar crystal. Sep 28 2023 pH 7.24 – 6.60
These sugar crystals are now gone.

What were the conditions for the highly viscous culture?
Treat prepared:
1. Added potato
2. Added phosphate
3. Increasing pH to 7.4 if not done

Some notes on TSP11, which presented highly "viscous" crystals.
First, TSP11 was a starch culture
One way precipitation to 6.0 (do not cross 7.5 threshold :)
Decant and separate
pH adj carefully upward to 7.4

And what may be the most important fact. TSP11 had equal blood cells from TSP01 added to it. This was quite possible to have produced the red viscous crystal formation that were so unique to TSP11.

So one of the things you are seeing here is that you seek to have almost a limitless number of culture variations that take place.

It is not real simple to produce the same
each time but you are beginning to see certain
levels of replication.

You do not have the yeast blood culture replicated
at that level w/ only one third of it but possibly
in between. Your combination of yeast blood and
advanced crystal likewise produced a phenomenal
result.

You should therefore expect a lot of variation in
the culture work but contend to see out
that which is reproducible.

It is still very worthwhile to see if you are
able to propagate TSP 01 into another

culture, namely pH 7.4 non starch peptone
7.4 starch sunday
Will do the next one well as incubated
and non incubated.

The means our forced culture is the TSP 30 series
No starch, peptone, pH 6.8

We will
1. Inject TSP01 into ~20 ml TSP 30 series
2. Raise pH to 7.4
3. One trial incubated = TSP 31
4. One trial not incubated = TSP 32

~~=TSP 32~~
~~=TSP 31~~

And then repeat for TSP 40 series =TSP 41
TSP 42

TSP20 ss to be discarded. Non productive.

You notice that TSP30 series precipitates exactly @ biological pH of 7.4. Seems far beyond coincidental.

What you can do down the road is accept a productive culture as representative of the TSP outcome. It does not need to be synth blood. Crystal biology is equally significant.

Then you apply your mitigation treatment. Then you back that up by UV & NIL.

This is a good strategy. Do not be concerned w/ the multitude of culture outcomes. Any productive culture, esp crystal biology coupled w/ CDB presence, is representative.

TSP12 WSB is turning pink on the dense sugar layer that has formed. Let's look. A needed word will be structure. It is thick, speaks of polymer again.

We see the transparent biological tube structure.

It appears to be a massive sheet of CDB & lysozyme & have no idea why it is pink. Blood not detected. Some protein structure. Incredibly dense & thick.

What characterizes TSP12 WS?

1. W/ starch
2. pH ~ 6.8 dropped from ~7.4 to 6.8
3. This is culture where globule clusters in sugar formed Oct 03
4. We know that it was precipitated by the pH set to 7.4

Ya we have two sets of TSP 12 w/ starch
They both have similar pH and do show similar
pinkish marine alga growth @ pH 6.65-6.8.

Oct 09 2023

The strategy now will be to adapt the cultures into petri dishes (recently available) to increase comparison productivity, reduce volume, & increase concentration, also leading to buffered trials.

For now, let's establish a visual overview of the base cultures in place.

In the future, the Pyrex vessels will be labeled as TSP Series X.0. Petri dishes will be individual trials.

Base cultures:

TSP01 - appears to be extinct. Synth blood appears to be extinct. Microscope analysis required to establish status. pH ~ 7.8 Appears to be a no starch culture. Minor surface activity.

TSP 11 - also appears to be extinct. Appears to be starch culture. pH ~ 7.5. Minor surface activity.

TSP-12 - WA With starch. pH ~ 7.4. Strong surface layer.
- It looks like I do have the synth blood again.
The might be where and why the pink color is coming in.

1. TSP12 - WSB pH 6.6? Set 1
2. TSP12 - WSB pH 6.8? Set 2
3. TSP12 - WSA pH 7.4

} all are starch cultures

I shall look @ these closely as the pinked surface layer is substantial and pink colored on each.

TSP 12 cultures seem to have been focused upon bringing the pH carefully (one way titration) to the biological pH of 7.4. They all have starch.
We see a note that there was

* NO FILTERING OR DECANTING past precipitation. Started cultures
* Careful titration of pH one way.
Oct 01 2023 had some notes on them.

Get the pH on these cultures.

1) TSP-12 WSA 6.5
BUT we see on 10/03/2023 the pH was set @ 7.4.

The way that it has become cowdenly more acidic and yet has been passable to synth blood production, and setting pH to 7.4 may have been a factor here.

2) TSP-WSB (Set2) 6.2 says pH was ~~set to~~ @ 6.8 on 09/30

3) TSP-WSB (Set1) Says on 10/03 pH was @ 6.65 measure 6.45

I need to:
Inspect ~~FSB~~ TSP-WSB (Set2) and TSP-WSB (Set1) as soon as possible and I need to split these cultures up as soon as possible and bring up to pH of 7.4. Need containers.

TSP12-WSB set 2 has almost no synth cells.
The texture of the surface layer of all three cultures is different even though they each have a pointed color.
We see that the pH measure 6.2 vs 6.5 for TSP12 WSA. This could be acidic enough to halt the process.

Let's look @ TSP-12 WSB set 1, also pink, but also of a different texture. Also no synth cells.
Let's buy the pH of both of these cultures @ 7.4.
& will not split them.

Synth blood feeding:

1. Biological pH assumed temperature
2. Gradual one way carpal pH transition 5.0 → 7.4
3. Heating of TSP OK, general jelling OK
4. upon precipitation, DO NOT DECANT. TSP remains cloudy
5. Maintain biological pH
6. Incubation uncertain.

Oct 10, 2023

I have the ham radio station in full operation in the lab now, both HF & 2m capability. Performance of station looks & has quite good.

One simple but important advantage of learning to construct your own buffer is to be able to calibrate the pH meter internally with the lab resources.

I am looking @ the TSP 12A w/ starch series that should themselves to be so interesting w/ apparent synth cells. I have split up the culture into two parts and have raised the pH to 7.4 for both.

Both cultures were thoroughly mixed on 9 yesterday.
~~SET 1~~ has already reestablished a ~~sl~~ slimy surface layer. TSP12WSA
~~SET 2~~ The split culture TSP 12 WSA (SET 10 P9) has the early stages of a surface layer appearing.

TSP12WSA shows less synth cells than yesterday but they still exist & anticipate both solutions to become more acidic w/ time. Then look for a diminishment in existence of synth cells as it relates to pH.
It is not impossible that a lower pH will become more favorable.

The are also now longer COB chains forming around the extremely dense mat of COB that forms the surface layer - these are blooming off filaments or chains form.

Here is what we see in TSP 12 WSA under the scope
Visually, on the surface layer: there are what appear to
be denser colonies on the surface. There are about four.
One of these colonies has also been examined under the scope.

They do show the same form as the previous "clot" reported.
Filament, protein, COB, synth ~~but~~ cell confluence.
I will let the mature and observe. All signs are, to-see,
however, that the synth cell replication is taking place.

TSP 12 WSB is an interesting case to report on. The surface
layer is richly developed w/ a contemiuous mat of colonies.
It even shows a light peat color. However, NO synth
cells show up in this culture, only massive COB concentration
and small crystals.

Recall that pH was set yesterday to 7.4 on all cultures
that are showing the peak surface layer colony development.

We now look @ last set of additional cultures that have been
under partial/moderate incubation over last 36 hrs.
1. TSP 41 w/ starch, precip, pH 7.4
shows some surface layer development.
2. TSP 30 "series" no starch precip, pH 6.8
show a bottom layer only.
3. TSP 31, no starch, precip, pH 7.4
shows bottom layer development only
4. TSP 40 series pH 6.8 established 10/05/23
presume no starch from color, pH 6.8, light surface layer,
dark bottom layer
5. TSP 40 series, pH 7.4, presume no starch, set 10/07
light surface layer, dark bottom layer.

This is the last time that I will see this culture set.
Surface layer observations under the scope: (Moderate incubation)

1. TSP 41, starch precip, pH 7.4 moderate incubation
   Moderately high level of CDB internal crystalline network. Crystal geometry moderate level of sophistication. CDB & star chain CDB. Homogeneous structure.

2. TSP 40 series pH 6.8 10/05 moderate incubation
   CDB, CDB chains (short), few large crystals

3. TSP 40 series 40 pH 7.4 10/07
   This is an interesting case. There is a new formation here. By color appear to be a no starch culture. Moderate incubation.

* For this case we appear to have protein clusters. In a vast number of these clusters there is a central dark circular structure w/ a central hole. The structures are quite large, expect in the order of 15-30 cm in diameter. They have never been seen before.

It is accompanied w/ CDB & CDB chains, large crystal development

Visually, the surface layer is no different than the TSP 40 series pH 6.8 10/05 Must quite different under the scope.

* The brings us to about 15-16 different culture products that we, continuing to exceed the ten reported on in a recent detailed paper.

I will save the last culture remnant because of the new feature showing. I will keep under moderate incubation.

All other remnant culture will now be discarded.

It would appear that the dozen + forms showing up can now be replicated w/ the culture variations. Spark cells still remain a priority until that feature is more often replicated.

—

I think we are now getting a handle on the culture products and variability. There is a fair degree of replication in place. There are very important steps to have in place.

We can now look at a representative developed culture form that can serve as a reference, especially for any mitigation or biochemical tests ahead.

—

Now let's start looking into buffers.
Acetic Acid - sodium Acetate is one
Citric Acid - Na Citrate should work.
apparently Citric acid alone w/ NaOH can work also
(ThoughtCo. com)

Bruce Thompson Chemistry Biology Forensic books are excellent
He has a full lab on acetic acid - sodium acetate buffer.

We can make sodium acetate by neutralizing NaOH w/ distilled white vinegar and evaporating to dryness.

Oct 11 2023

Let's develop a buffer that today.
We have an order.

1. Borax
2. Sodium Acetate
3. Ammonium Acetate
4. Washing Soda (Sodium Carbonate)
5. Ammonium Sulfate
6. Potassium Permanganate
7. Oxalic Acid

You have 3 citrates available.
Sodium citrate (pure)
Calcium citrate (supplement)
magnesium citrate (supplement)

It looks like our best option with what we have now
is citric acid and sodium citrate.
2 Methods given by thought co.

1) 2.1 gms citric acid in a liter
Let's use 300 ml.
Mass of vessel = 243 gms
(2.1 gms)(.3) = 0.63 gms
Dissolve 0.63 gms. in 290 ml H_2O.
243 gms + 290 ml = 533 gms
Bring pH up to 6.0 w/ NaOH-KOH
Bring final volume up to 300 ml.

Now Khoshtoo says you have two ways to make the buffer. The first requires citric acid + sodium citrate (I have these).

The second method says only citric acid is required.

In both cases, the final pH is brought up to 6.0. I am now trying the second method. Right now I have no idea how the second method can work w/out the conjugate base.

Let's try & see what happens. For the first method, the reactions

$C_6H_8O_7 + NaC_2H_3O_2 \rightarrow$

≈

$C_6H_8O_7 + 3NaHCO_3(aq) \rightarrow 3H_2O(l) + 3CO_2(g) + Na_3C_6H_5O_7(aq)$

or

$C_6H_8O_7 \cdot H_2O + Na_3C_6H_5O_7 \cdot H_2O \rightarrow$

So we are using that sodium citrate has different forms. Our packet just says sodium citrate.

Citric acid is a tribasic weak acid. (not sure what this means)
Citric acid is a triprotic acid H_3cit (Easier to understand)

There is quite a bit going on here. Citric acid is triprotic which also complicates the case considerably. Lehninger Texts. only has a thorough discussion on what happens when you added NaOH to citric acid and how it acts as a buffer.

What you see is that the titration plot shows you what is happening and why, or at least, how Citric acid does act as a buffer as shown w/ the titration of Citric acid w/ NaOH.
It has a very shallow plot change between pH 2.5 to 5.5 and that encompasses two equivalence points. Break away really does not happen until you pH exceeds 5.5.

Furthermore, it has an excellent plot of phosphoric acid so we definitely should use this, covers a pH range from ~1.5 to ~13 pH.

Titration curves show a lot

We see in our Can that we are at pH 6.0.
This mean that citric acid will now break away to the 3rd equiv point @ pH ~10.

Let's go ahead and titrate to 6.0
Also what is the concentration of our citric acid before proceeds?

Our molar concentration is 0.011 M via Chemex. As it is very weak.
It will take very little NaOH to reach the equivalent point.

The exercise could have demonstrated as easily w/ 100 ml vs 300 ml.

Ok, the wa a great exercise.

1. Small volume can be used to demonstrate the point
2. Citric acid alone, without even using sodium citrate (conjugate base) acted as a rather effective buffer all the way up to pH ~ 7.0. It is why the that the titration curve flattened steeply. Is the apparently is a unique case that thought it recognized and provided that simple option. Apparently it has to do with the fact that citric acid is hygroscopic.

Along w/ the ~~clemtext~~ labwetext discussion on using the same procedure w/ phosphoric acid it shows you how powerful titration is in the identification of an acid.

We need to get on the titrating our acid as soon as possible. The hypothesis is indeed phosphoric acid.

Also we ask, how do you identify the equivalence point(s) on a titration? Apparently it is midway between the slope breaks.

Now we realize that we want to separate off the our acid means we do want to ~~do~~ count those products in the case.

I will run two titration trials. We will use two equal volumes. One will be the native form of the TSP, the other will be decanted TSP of the precipitation and pH = 7.4

NO STARCH TRIALS

You are not after concentration, just the profile of the titration curves.

I added ~10 ml of strong KOH-NaOH to precipitate the TSP in one sample @ a pH of 7.4 so concentration are different. This culture is a ~~starch~~ NO version

Now we already know that the pimp version is diluted by a factor of $\frac{90ml}{80ml} = 1.125$

Next step is to bring the same solution down to as close to 2 as possible

25

Let's set volume to 30ml each.

Decant beaker mass = 33.70gms

33.70 + 25.0 = 58.70gms

Decant native TSP beaker mass = 34.98gms

34.98gm + 25.0 = 59.98gms

Will do titration w/ strong H_2SO_4, most likely at ~ 10M. See if I can now bring to pH down to 2.

Initial Volume H_2SO_4 = 20.0 ml

Actually, down to 3.5 ml is sufficient. We already saw the steep drop from pH ~ 7 to 3.5

The already matches the first equivalence point of phosphoric acid.

Let's keep volume disturbance during this stage to a minimum.

Current burette volume = 18.1 mL ⇒ Δ = 1.9 ~ 10M H_2SO_4 added to lower pH from 7.2 to 3.42

This is the decanted version. Next we will titrate the native TSP version down to a lower pH ~ 3.5 also
Initial Volume Burette = 18.1 mL current pH = 5.50

Final burette volume 16.8 mL. Δ = 1.30 mL
make sense because you start @ a lower pH.
Initial pH = 5.50 Final pH = 3.41 Δ = 2.09
To calculate rate:

Decant Avg pH rate : $\frac{7.2 - 3.42}{1.9\ mL} = \frac{1.99\ \Delta pH}{mL}$ Native: $\frac{5.50 - 3.41}{1.30\ mL} = \frac{1.61\ \Delta pH}{mL}$

Ok, now we go to the direction & create the titration profile. We will use the strong KOH – NaOH.

This is quite interesting work. You have the real prospect here of identifying (possible repetitions of) the acid forms in the TSP

TSP Decanted Farm Titration
(Post Precipitation) No Starch
Beaker Mass Σ = 61.35 g mis

Burette Volume (ml)	pH	Δ	ΔRate
20	3.43		
19	3.43		
18.5	3.46		
18	3.48		
17	3.58		
16.3	3.67		
15.9	3.77		
15.4	3.88		
15.1	4.02		
14.9	4.30	.28	1.4
14.8	4.61	.31	3.1
14.7	5.15	.54	5.4
14.4	6.04	0.89	3.0
14.1	8.12	2.08	6.9
13.9	8.52	.4	2.0
13.75	9.00	.4	3.2
13.3	9.72	.72	1.6
13.1	10.11		
12.85	10.25	10.32	
12.4	10.32	10.42	
11.9	10.36	10.54	
10.9	10.55		
10.3	10.56		
9.9	10.56		
9	10.56	Max Reached.	

X = 7.08 ← Equiv. Pt.

Here our equivalence point occurs @ pH 7.00
The corresponds to a volume of 14.25 ml
The mean actual volume consumed is 20 - 14.25 ml
= 5.75 ml

Now half equivalence point is 5.75/2 = 2.875 ml

and 20.0 - 2.875 ml = 17.125 ml

$\Delta = .125(3.58 - 3.46) = 0.013$ so the corresponds pH = 3.57
~~17.013 ml and 20.0 - 17.013 ml =~~
and volume corresponds 17.125 ml
@ half equivalence point relation is $[A^-] = [HA]$

pH = pKa from Hasselbach equation

3.57 = pKa

$Ka = 10^{-3.57} = 2.69E-4$

Formic acid	1.8E-4	$HCHO_2$
Hydrofluoric	6.3E-4	HF
Lactic acid	8.3E-4	$HC_3H_5O_3$
Nitrous	4.0E-4	HNO_2
Uric	1.3E-4	$HC_5H_3N_4O_3$

This value is in error → pKa = 3.96E-6

Our closest candidate in the given report question and most logical is nitrous acid. Nitrous or acid is a conjugate acid of a nitrite. Decrementing busy function.

But all are candidate now. Now work on the nature for

Oct 13 2023

Obviously the TSP is quite complex w/ these ~~four~~ 1½ dozen physical + protein manifestations.

1. You are seeking a "representative" active culture from that can be replicated

2. Then seek to identify the acid(s) that are in the TSP. Can it be more than one? Prior prep and post prep? What happens in titration w/ a mixture of acids? Could there have been metabolism of a polyprotic acid w/ a mixture?

3. You can see once again how chromatography can easily apply here, as applied to even the most apparently "pure" product, eg a decanted precipitated TSP that has been brought up to pH 10.4 and become very transparent as the max insolubility was identified before.

5. What does UV say w.r.t. the 10.2 precipitated decanted TSP? Protein still show up?

6. Nitrous acid Candidate Under investigation. Conjugate base → nitrate. Can test strips for nitrate you need for that here?

1. A new steady state incubator is in place now, slow crack cooler on warm setting.

8. Review the Exotic Culture set a as:
   1. settle in on a repeatable active culture form
   2. Work on acid identification
   3. Crude Chromatography for the separation
   4. NIR and electrochemistry will need to be brought in here.

9. Paper Candidate:
   1. Human & synth blood difference
   2. "Nanotechnology" - Gateway in Chemistry and Molecular Analysis

10. Mitigation & Containment:
   1. Bi Carbonate influence w/ Enzyme?
   2. Buffer Analysis of Culture behavior

11. Here is a question: Is it possible that TSP01 was actually brought up to pH 10.2 and was only titrated since we see they are both transparent. Is it possible it was @ the high pH level and then acidic production over 2 weeks of time decreased to pH to 7.2 w/ Crystal development very unusual geometry and synth blood appears inert

I will start to monitor the question #11 & Consequently monitor the pH of the seed residual titrant C pH 10.56
The question is whether the pH decreases over time.
There is some question because of clarity of the residual titrant as to whether TSP 61 had the additional titration applied to it.

Now, the test for today is whether the decanted TSP titrates differently than the native TSP. All titrations right now are being done w/ no starch cultures. I may be in the process of shifting over & predominant starch cultures due to the extreme and unwanted growth developing in TSP-12 WSA.

Our goal is to seek identification of oxide w/ the the culture, post precipitation lab comparison of native and decanted forms.

We see from Oct 12 notes that we already brought the pH of the native TSP down to 3.41 so it is supposed to be ready to titrate. It has been sitting for 24 hrs so lets check the pH.

TSP Native Form Titration - No Starch
No precipitation applied. Native form w/ pH dropped from ~5.5 to ~3.4

Burette Volume	pH
19.9 ml	3.30
19.7	3.30
19.1	3.31
18.9	3.33
18.4	3.35
17.9	3.40
17.2	3.48
16.8	3.59
16.2	3.76
15.8	4.06
15.4	4.56
15.2	5.25
15.0	5.48
14.9	5.90
14.8	7.20
14.5	7.55
14.2	7.89
13.9	8.35
13.7	10.03
13.2	10.14
12.9	10.20
12.6	10.25
12.1	10.29
11.7	10.32
10.9	10.34

Gran titration shows equivalence point at 6.7. My estimate is @ 6.55 very much within limits.

Equiv Pt = (19.9 - 14.85 ml) = 5.05 ml pH ≈ 6.55
½ equiv pt = 2.525 ml → 2.525 + 19.9 = 17.375 ml
pH = 3.46 $10^{-3.46}$ = Ka = 3.5E-4
vs 2.69E-4 on Decant Titration $\bar{X}$ = 3.095E-4
≈ 3.1E-4 Nitrous Acid remains best candidate as monoprotic acid

However! 2nd equiv. pt here
@ 13.8 ml - 19.9 ml = 6.1 ml ½ equiv pt = 3.05 ml
19.9 - 3.05 ml = 16.85 ml pH = 3.661
Ka = $10^{-3.661}$ = 2.18E-4

Now, looking for candidates...

Methanoic acid Ka is 1.8E-4

$HCO_2H \rightleftharpoons HCO_2^-$ Methanoate ion

Now the source, sigma aldrich lists Ka for nitrous acid 7.2E-4 ⇔ nitrite ion

vs our alternate source that said 4E-4.

Methanoic acid is listed as the last entry in the strong acid category.

Thought Co:

Formic acid is also 1.8E-4 $HCHO_2$

~~4E-4~~ HNO_2

Nitrous Uric

NO

1.3E-4 $HC_5H_3N_4O_3$ How and why did they say this?

This is wrong! $Ka_1 = 3.98E-6$

This cannot be true ← ? !

$pKa_1 = 5.4$

$pKa_2 = 10.3$

What my data seems to be saying is that I get the same acid Ka regardless of whether I use the decimal or the notation format.

On average of all three values

Thought Co makes a significant error here

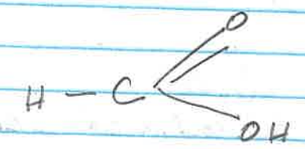
2.69
~~1.8~~ 3.1 } E-4 = 2.66E-4
2.18

From sigma aldrich table they really want anything else E. Complete. Methanoic acid = 1.8E-4

3 different ways I end up @ the same value.

Formic acid is indeed the same thing as methanoic acid.
Methanoic acid is the simplest Carboxylic acid

HCOOH



It is
"An example of a protic solvent w/ high acidity"
It is a colorless, corrosive, pungent liquid carboxylic acid.
It occurs in stinging ants, nettles, pine needle & insect.
Venom of bee and ant stings
At ambient temperatures it has a strong penetrating odor
like acetic acid. Miscible in water.
Formic acid is strongly acidic. 1mM pH = 3.47
mM means milli molar ie 10^{-3} mole/liter
Strongly acidic therefore
.001M = 3.47 pH
.01M = 2.91 $\Delta = .56$ $\bar{x} = .55$
100M = .01M = 2.38 $\Delta = .53$
So estimates are
.0001 $\cong$ 4.02 pH
.00001 = 4.57
.000001 $\cong$ 5.12
.0000001 $\cong$ 5.67 $\equiv$ 1E-7M concentration ~~estimate~~
Corrosive to metals and tissue.
"
Under normal circumstances, the formic acid in our body is too dilute to be dangerous".

Formic acid is an irritating chemical present in the sprayed venom of some ant species and stinging nettles.

If concentration is high:
1. Burns and blisters on the skin
2. Injure eye and mucous membranes
3. Can affect breathing
4. Prolonged exposure - liver or kidney damage

Formic acid may have played a role in the origin of life on earth. - Amino acids - nucleotides

Higher levels of formic acid in urine may be a biomarker for impaired cognition. May serve as an early detection for Alzheimer's disease.

Alzheimer's is expected to double by 2050.

Could be highly important and likely could be tested for years.

Oct 14 2023

The identification of the acid(s) in the TSP is to be a very significant accomplishment. Acid-base chemistry is obviously driving many of the protein expressions & products. It is likely to become very important also as mitigation strategies develop further.

1. There is a separation between starch and non starch cultures, although in general it appears that the starch based culture will be to greater advantage.

2. There is a separation to be made based upon pH. At this point, there appear to be three primary levels of interest:

pH ≅ 5.6 - 5.8 Native TSP (filtered from culture, no additional processing)

pH ≅ 7.4 Highly important biological pH level. There the whole Cultus growth & development appears to be most active and advanced.

pH ≅ 10.2 Maximum molecular level of the TSP. Preliminary molecular deterioration affects interpretation of charge & apparently acid identification within the native TSP portion.

Some topics emerging on the preliminary identification of methanoic (formic) acid:

1. The simplest Carboxylic acid there is
2. Confirmation of identity
   NIR show C=O bond?
3. Conjugate base of formic acid?
4. Titrating of formic acid vs a polyprotic acid?
5. Determination of acid identity in native state vs precipitated and decanted?
   Two separate equivalence points leading to the same identity apparently dependent upon the native vs precipitated/decanted state?
6. Does UV show proteins in either/both native vs precip form?
7. Urine analysis now becomes paramount. Formic acid in urine is a biomarker for Alzheimers...

   My urine sample has a pH of 6.03
   How does it titrate?

Chris Titration: Initial pH 6.03
w/ NaOH - KOH

Burette Volume	pH	
19.8	6.04	I am able to get MathCad 15 working
19.7	7.42	on the HP Windows 8 system
		Very useful. Can we get on the gateway?

So that did not work. You need to use a much more dilute version of NaOH-KOH
We see, however, that it did precipitate the iron to some level. pH of titrant is 10.50

19.4	6.08	Ok, please what we see in the
19.35	6.22	titration curve. You need to
19.20	6.43	acidify the solution further
19.0	6.80	before starting. You are already
18.9	7.02	at the midst of the climb when
18.75	7.26	you start @ pH = 6.08.
18.62	7.53	The ph is on a steeper rate of
18.3	7.81	climb until pH ~ 8.3, then
18.2	7.98	it levels off. Let's acidify
18.0	8.11	to eliminate ~ pH 3.0.
17.95	8.20	
17.90	8.28	Ok, I have the pH down to
17.65	8.39	~ 3.63 w/ H_2SO_4.
17.2	8.50	
16.9	8.66	Let's go again
16.0	8.85	
15.0	9.16	
14.1	9.45	
13.4	9.64	
12.5	9.81	
10.9	9.99	
8.85	10.12	

Urea Titration

Burette Volume	pH
19.25	3.57
18.8	3.57
18.0	3.58
17.1	3.59
15.8	3.63
14.7	3.71
13.3	3.86
12.3	4.08
11.8	4.31
11.3	4.62
11.0	5.09
10.8	5.65
10.65	6.02
10.4	6.49
10.1	7.05
10.0	7.83
9.9	8.33
9.7	8.64
9.2	9.29
9.0	9.66
8.8	9.97
8.35	10.09
7.8	10.18
7.0	10.24
6.3	10.28

We will do the ag ain because we varied Concentration of KOH-NaOH inadvertently. Results will be close but we will repeat.

Our steepest portion seems to be @ x = 9.95 mL w/ pH = 7.44

9.95/2 = 4.975 mL
pH = 3.71

$10^{-3.71} = 1.95E-4$

Well well well
formic acid is 1.8E-4

Among all values our formic acid, our average is now:
2.69E-4
3.1E-4
2.18E-4
1.8E-4
$\bar{x} = 2.44E-4$
vs 1.8E-4 Methanoic

6.3E-4 vs Hydrofluoric
8.3E-4 Lactic
7.2E-4 Nitrous
1.3E-4 Uric

Now certainly urea acid seems to be a contender here.

Ok, so the plot thickens here.
The composition of urea acid is significantly more complicated in urea acid vs formic acid. It is far more likely that the culture is producing a much simpler organic acid, namely formic acid.

However, in urine there is indeed urea acid. A certain level is anticipated. Our pKa is likely ~~w/in experimental error determination of~~ No
~~acid. Since the pKa's are so close~~ WITHIN urine, ~~it would be difficult to separate out~~ not!
urea acid vs formic acid. But not to much
the case as far as the culture is the case.
The likelihood likelihood of formic acid in the
culture is quite high, especially since the bias
of all measurements is to the opposite.

However, we can not make such a claim w.r.t
to uric acid. They are too close to separate. Also
we must assume though that urea could easily
contain both urea acid and formic acid and
it would be almost impossible to distinguish
w/out additional methods.

But in either case, the CDB would add to the
acidic load in the body. Just a coincidence
that formic acid is produced by the CDB - certainly
makes more difficult to distinguish.

A very interesting case.

You would need to correlate of the factors such as CDB concentration in the blood of the expectation of formic acid presence.

Mixture of two acids - influence upon titration?

Process of formation of formic acid vs that of uric acid is important to consider here

6.0 - 7.5 Normal pH range for urine.
Someone else says from 4.5 to 8.
How is that for inclusion?

NIH gives 5-7, therefore $\bar{x} = 6$

Ok, what are the conjugate bases?

Methanoic acid? Formate (methanoate)

Uric acid? Urate?

Also the equilibrium point is different.
My measured equilibrium point of formic acid is 6.55. Go table source has stated as 6.7. I also measured 7.08

Take a look at this

Uric acid equilibrium point is 6.5-6.8

My look too close to call.

Indeed the plot sat pretty thick on the one.

Another way to seek distinction is to precipitate arsenic up to 7.4 and compare the precipitate against the TSP precipitate.

I have MathCad working on Gateway now. This is a great program for me w/ history & I analysis, matrix operations, etc.

Oct 15 2023

1. Titrations will need to be repeated to the point of replication.
2. Comparison of urine precipitate @ pH 7.4 w/ TSP precipitate @ 7.4 by NIR, UV - do they share common ground?
3. Urine ~~precipitated to~~ alkalinized to pH 10.2 produces a deep orange color w/ a significant amount of precipitate. How does the layer compare w/ TSP precipitated to 10.2?
4. Method of formation of uric acid by the body vs the method of production of formic acid by a culture.
5. Any tests of distinction between formic and uric acid?
6. Molecular weight of TSP and its precipitate / decant version?
7. UV spectrum of folic acid, uric acid available?
8. ✓ Order folic acid - Done

Uric acid is a product of the metabolic breakdown of purine nucleotides. It is a normal component of urine. - Basically per nucleotide are the monomers of DNA.
Purine is a heterocyclic aromatic compound. Found in high concentration in meat, and esp. internal organs such as liver or kidney.

Dried beans, peas and beer have high content of purines also.

Ok, the info on uric acid is important. Uric acid is a totally different animal.

Uric acid is a diprotic acid with $pKa_1 = 5.4$ and $pKa_2 = 10.3$

Hardly anything close to monoprotic formic acid w/ $pKa = 3.75$

At physiological pH urate predominates in solution a heterocyclic compound

The water solubility of uric acid and its salts is LOW. The solubility of uric acid and its salts in ethanol is VERY LOW or negligible negligible. There is likely the basis of a useful distinction.

$$pKa = -\log_{10} Ka$$

$$Ka = 10^{-pKa}$$

so for uric acid, we should have $Ka_1 = 10^{-5.4} = 3.98E\text{-}6$

$Ka_2 = 10^{-10.3} = 5.01E\text{-}11$

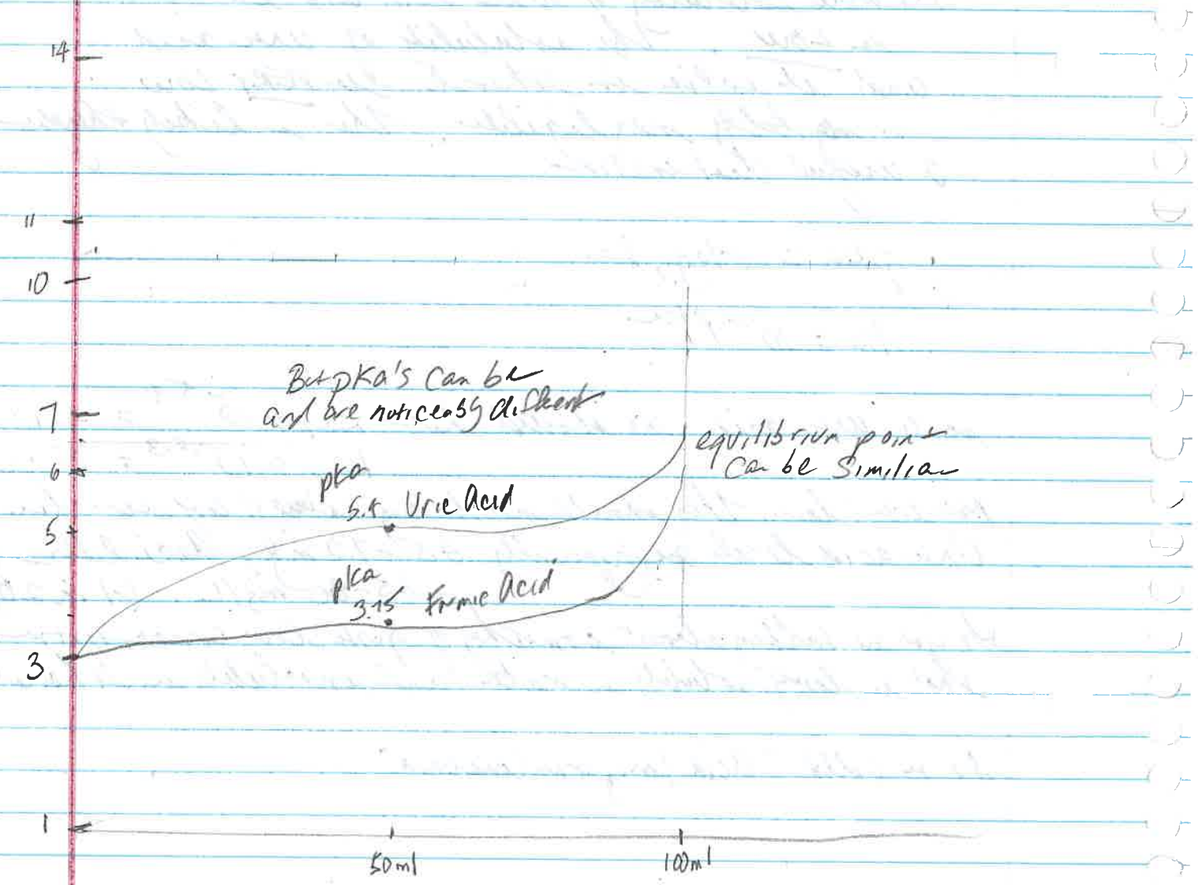
Not even close. There should not be any issue of conjugate base. Uric acid levels are typically 2.5-7.0 mg/deciliter (100 ml) or 25-80 mg/L. (~1 in 20,000)

So you are talking about something of quite low concentration that is barely soluble in water and insoluble in ethanol.

It is called "Acid Ionization Constant"

Urea acid titration behavior as well as solubility behavior both between formic acid and urea acid should not ever be close to one another,

We can construct good models of the titration curves w/ the pKa values alone, let alone monoprotic & diprotic. Now do realize that the equivalence point of urea acid would be quite difficult to acquire since the pKa are so shift @ 10.2. Nevertheless we expect



Some very useful comments here on uric acid esp related to solubility & pH.

Chapter 165 Uric Acid

https://www.ncbi.nlm.nih.gov/books/NBK273/

Walter G. Barr.

Definition

Uric acid is the ultimate catabolite of purine metabolism in humans and higher primates. It is a weak organic acid that under physiologic conditions exists mainly as a monosodium salt. At a pH less than 5.75, as may occur in the urine, the predominant form is nonionized uric acid. The solubility of monosodium urate is about 18 times greater than uric acid in aqueous solutions. This solubility differential provides the therapeutic rationale for alkalinization of the urine pH to greater than 6.0 in patients forming uric acid stones.

The upper limit of plasma uric acid may be defined by a statistical range in a normal population. Epidemiologic studies in the United States have generally accepted 7.0 mg/dl as the upper limit in adult men and 6.0 mg/dl in women.

The physiochemical definition of hyperuricemia may be considered 7.0 mg/dl measured by the specific uricase method. This represents the solubility limit of urate in plasma at 37°C. Levels beyond 7.0 result in supersaturated solutions that are prone to crystal formation.

Uric acid levels are influenced by age and sex. Prior to puberty, the average serum uric acid is 3.6 mg/dl for males and females. Following puberty, values rise to adult levels with women typically 1 mg/dl less than men. This lower level in women apparently reflects estrogen-related enhancement of renal urate clearance and disappears at the menopause. Many additional factors, including exercise, diet, drugs, and state of hydration, may result in transient fluctuations of uric acid levels.

Technique

Currently, two methods are widely utilized to quantify uric acid. A colorimetric method depends on the reduction of a chromogen such as sodium tungstate by uric acid to produce a measurable color change. This technique has been commonly employed in automated hospital screening (SMA systems). The method measures materials other than urate, such as ascorbic acid. Colorimetric determinations are generally considered an overestimation of true uric acid levels, and the normal range is usually 1 mg/dl higher than the more specific enzymatic techniques.

Enzymatic determination of uric acid results from the specific oxidation of uric acid by uricase, which converts its substrate to allantoin. The differential absorbance of these substances at 293 nm allows quantification.

$$\underset{\text{(absorbs at 293 nm)}}{\text{Uric acid}} + 2\,H_2O + O_2 \xrightarrow{\text{uricase}} \underset{\text{(nonabsorbing at 293 nm)}}{\text{Allantoin}} + H_2O_2 + CO_2$$

Although traditionally a more expensive technique, uricase methods are currently available for SMA systems at comparable costs and are gradually replacing the less specific colorimetric method.

Basic Science

I have ordered formic acid - this will be a useful reference.

At this point everything we say say that I have isolated and identified methanoic acid, ie, formic acid in the TSP.

I also appear to have done so in urine. There should be no real confusion between formic acid in the TSP and Uhead, vs Uric acid in the normal urine. Because of

1. Concentration levels
2. Solubility behavior
3. Equilibrium constants and pka values
4. pH / solubility behavior

There are extremely significant features here.

Let's look @ relative molar concentrations

We already have a rough estimate of our formic acid molar concentration from Oct 13 notes as 1E-1. Rather very rough but it is a starting point.

We will need to learn & relate molar concentration to pH very soon.

But the next question is, what is the approx molar Concentration of urea acid in urine.
Assume we have 50 mgs/L.
Uric acid MW = ~~154.105~~ gms/mole OK @ 168.1 gms/mole

$C_5H_4N_4O_3$ (168.1 gms/mole)

$\frac{50E-3\ gms}{168.1\ gms/mole}$ = 2.97E-4 molar

$\frac{50E-3\ gms}{1000\ ml} = \frac{168.1\ gms}{x}$ x = 3.36 E6 ml

$\frac{168.1\ gms}{1\ liter}$ = The above not noted expectations actually came out much lighter than anticipated.

$pH = -\log[H+]$ $H+ = 10^{-pH}$ M

M is molarity, moles/liter m is molality, moles/kg

Formic acid 1E-3M = 3.41 pH
1E-2M = 2.91

We can theoretically estimate our corrected molar concentration of formic acid knowing our pH value and the acid ionization constant.
pH ≈ 5.8
Formic Acid $K_a = 10^{-3.75} = 1.78E-4$

From an online calculator, it should be on the order of 2E-6M concentration. H+ = 1.978E-6 M

The online calculator of pH for acids is very interesting and useful to have. It even happens to have an example using formic acid.

It solves for the ionization equation.

$$HCOOH = HCOO^- + H^+$$

$$K_a = \frac{[H^+][HCOO^-]}{HCOOH}$$

when $K_a = \frac{x^2}{C-x}$

C is the molar concentration of the solution

x is the molar concentration of H^+

And once you have H^+ then

$pH = -\log([H^+])$ by definition

$-\log$ is needed because the concentration is < 1.

There is a very useful calculator

If we use the Calculate and assume a pH of ~5.7
for our TSP and further assume that the acid
involved is formic acid, then we have
$K_a = 10^{-3.75}$

Given $K_a = 1.78E-4$ pH = 5.7 of our TSP
then our concentration of formic acid is: ~~2.2E-6M~~ 2.0E-6M
our concentration of H^+ is: 1.995E-6 M

The says @ the level of concentration the acid is essentially
fully dissociated.

If the pH is much lower, then is not the case at all
and a much smaller proportion of the acid is dissociated.

Now for Uric acid (it is diprotic)
We have
$K_a = 10^{-5.4} = 3.98E-6$
Now if the urine pH is ~6 then
our Concentration of uric acid is ~ 1.25E-6M
and " " $[H^+]$ is ~ 1E-6

Then the Concentration levels of the acid would be
in the same order. But pka's are
significantly different, 3.75 vs 5.4

Ok, so that urine as an is an improvement
on Concentration of NaOH - KOH Stable.

Ok, another source confirms the pka of uric acid.

pH urine ~ 5.75 in blood
5.35 in urine

this confirms the ka as $10^{-5.75}$ = 1.78E-6 blood,
$10^{-5.35}$ = 4.47E-6 urine

There is simply no way that formic acid can be compared by weight with uric acid and the Thought Co. com value for ka is erroneous. Multiple sources show conjugate pka of uric acid on the order of 5.5, not 3.75.

So lets acidify urine again and titrate once again. Acidify w/ H_2SO_4 to ~3 pH

The question is what strength base do you want to use to titrate? I think that we should be able to use our NaOH-KOH base @ a level of ~30% concentration. Let's try it.

Mass of vessel 354gms. Add NaOH-KOH to 400gms
Δ= 46gms (3) = 138gms
(H_2O) 138gms + 354gms = 492gms total

Initial pH of urine sample 6.05

Repeat Urene Titration

Burette Volume	pH
19.4 ml	3.50
18.8	3.51
18.0	3.52
17.3	3.54
16.2	3.57
15.2	3.61
13.7	3.68
12.0	3.80
10.2	3.97
9.0	4.18
8.4	4.32
8.0	4.48
7.8	4.66
7.5	4.88
7.0	5.22
6.9	5.44
6.75	5.74
6.3	6.29
6.1	6.65
6.0	6.99
5.8	7.56
5.5	7.90
5.15	8.24
5.0	8.46
4.4	8.83
4.1	9.25
3.7	9.66
3.1	9.87
2.3	10.02
1.5	10.11
0.4	10.17

This was a perfect titration in terms of titrant range.

Our estimated equilibrium point is @ Vol = 13.6 ml
pH = 7.56

1/2 equilibrium volume = 6.8 ml
pKa pH = 3.76

You cannot get any better than this.
pK Ka = $10^{-3.76}$ = 1.74E-4

VS theoretical value of 1.8E-4.

This is an excellent confirmation of formic acid being found in the urine.

Uric acid requires a pKa of ~5.4 that corresponds to a vol of 12.5 ml
2(12.5 ml) = 25 ml which is completely beyond the titration and not even close to the equilibrium point determined.

The was a urine titration w/ a proper concentration of OH titrant.

The Confirm formic acid in the urine sample. The the main implications needs to match the TSP identification.

Next we titrate the TSP again

We now add acid neutralization as a methods strategy

1. Balm - balancing act
2. Ultrasound - balancing act
3. Enzyme - protein disruption — BOTH BLOOD & URINE BUFFERING CAPACITY MIGHT
4. Acid neutralization. Bicarbonate - Citrate buffer. Pancreas is a primary target.
5. Methylation
6. Antioxidant of Cancer, NAC, alpha lipoic, all vitamins

The is a major step forward.
Certainly time to look @ blood again.

We can still compare precipitate a NIR

Our next titration is a natural, native format TSP culture (filtered). Native pH ~ 5.7 ✓ Now acidified w/ H_2SO_4 to ~ pH 3.5. Titrant is 30% NaOH - KOH

Burette Vol	pH				
~~19.3~~	3.40		2.6	8.23	
~~18.2~~	3.40		2.2	8.39	
17.6	3.41		1.8	8.55	Our average Ka of both urine & TSP titrations is 1.94E-4 vs 1.8E-4 Quite consistent work.
16.55	3.44		1.2	8.75	
15.6	3.46		~~0.85~~	8.94	
14.4	3.57		0.0	9.36	
13.3	3.56	13.35 →	0.0	9.36	
12.2	3.61	12.90 →	-.45	9.83	
11.2	3.67	12.1 →	~~-1.25~~	10.03	
10.3	3.73	11.3 →	~~-2.05~~	10.12	
9.4	3.81	10.2 →	-3.15	10.19	
8.6	3.90	8.7 →	-4.65	10.25	
7.9	3.99				
7.0	4.12	Est. Eq. Point		16.15 ml	
6.7	4.24			pH 7.70	
6.0	4.39				
5.65	4.66	½ eq = 8.075 ml			
5.1	5.13	pH = 3.67 = pKa			
4.82	5.54				
4.40	5.95	Ka = $10^{-3.67}$ = 2.14E-4			
4.0	6.47	vs 1.0E-4			
3.8	6.82				
3.65	7.23				
3.25	7.67				
3.0	7.97				
2.8	8.10				

Once again, we demonstrate the existence of Formic Acid as the acid in the TSP (native format) as well as in urine.

Extremely consistent work here on place.

Oct 16 2023

I now have Mathcad 15 working on three computers. Very helpful and now prohibitively expensive otherwise. Very fortunate to figure out how to get my version running.

The incubator pulsed version heat on low - perfect for drying. Low setting works perfectly for incubator.

I have two more computers to try to get the NIR software working in the lab. It has failed on three so far. Five computers in the lab.

I have prepared a NIR card of the TSP native form, w/ starch, fully titrated to pH 10.2. Precipitate.

I am now working on preparation of a urine sample, determined to contain formic acid, to compare the pale precipitate that forms to that of the TSP above precipitate. Titrations of urine appear to behave exactly the same as the TSP w/ the white precipitate settling to a bottom layer.

I need to look at the formic acid - Carbonate ion relationship. Also the citric acid - Carbonate relationship (buffer) as in Alka seltzer.

Need to look @ blood & culture status.

OK, sure enough. Citric acid + Baking soda does make a buffer.

"Sodium bicarbonate does act as a ~~base~~ neutralizer for base" [actually, acid? eg, Carbonic acid]
See Oct 17 2023 Notes

formed sodium citrate acts as antacid.

This is perfect and it explains the success in the early cultural work w/ sodium + calcium citrate years ago
Now we know what the acid is and why it would work

Bicarbonate ion and citrate ion should act as buffers
Both bicarbonate & citrate together likely & the ~~citrate~~ [Identical, these compounds - Oct 17]

$3NaHCO_{3(aq)} + H_3C_3H_5O_{7(aq)} \rightarrow Na_3C_3H_5O_{7(aq)} + 3CO_{2(g)} + 3H_2O$

FIND THIS SOURCE AGAIN (DOC) (STORED)

The sodium citrate acts as an antacid.

$Na_3C_3H_5O_{7(aq)} + HCl_{(aq)} \rightarrow H_3C_3H_5O_{7(aq)} + NaCl_{(aq)}$

ALSO EXERCISE PHD ARTICLE

Excess sodium bicarbonate from the alka seltzer acts & neutralize base: [(vs acid like Carbonic acid?) See Oct 17 notes]

$NaHCO_{3(aq)} + NaOH_{(aq)} \rightarrow Na_2CO_{3(aq)} + H_2O_{(l)}$

I cannot seem to get the NIR software to work in the lab. [But you can transplant collected data to lab easily for analysis there]

There are no obvious ~~similar~~ similarities or differences between the TSP native format w/ starch precipitate and the urea precipitate. If there is a difference, it may center on a lack by a decrease of hydrocarbons (e.g. methyl, methylene groups) in the urea precipitate vs the TSP precipitate. I do not see any strong conclusions that can be made from the comparison @ this time. Certainly it can not be stated w/ confidence @ the time that the precipitate forms are highly similar. This is important to know. There is no real reason to expect them to be the same as the urea will be complex and clustered in many ways.

Just made a ~~file~~ became aware that it is quite easy to zip a folder and send by email in Windows. This is very useful and will eliminate most of the hassle of NoNIR software in the lab. Raw CSV files or the OPUS file(s), folder, etc can be easily sent now to the lab and analysis can take place there.

No need now to analyze NIR data or integrate at the newer computer, only collect the data.

Oct 17, 2023

I will conduct a separate individual's urine titration. The sample is very clear and pH is 6.4 so it would appear to be in a more favorable state. Will watch for signs of precipitation upon any pH change. Hypothesis is that the sample has a lower formic/uric acid content than the previous samples. Concentration determination of acid level will therefore become more important. Will need pure NaOH. Will acidify to ~3.5

There are no VOC's from current culture. Anticipate it is complete. No – VOCs @ 20 ppb

2nd Individual Urine Sample

Burette Volume	pH	Vol	pH	Vol	pH
20.0	3.55	3.0	4.66	19.0 =0.00	8.44
19.7	3.55	2.9	4.75	18.8 -0.2	8.65
19.0	3.55	2.55	4.94	18.4 -0.6	8.84
18.2	3.56	2.20	5.13	18.0 -1.0	9.23
17.0	3.57	2.00	5.33	17.75 -1.25	9.60
15.8	3.59	1.95	5.49	17.3 -1.7	9.84
14.3	3.63	1.87	5.72	17.0 -2.0	9.99
12.1	3.70	1.75	5.91	16.70 -2.3	10.09
10.0	3.80	1.60	6.21	16.0 -3.0	10.17
7.6	3.95	1.20	6.50	15.65 -3.35	10.21
6.9	4.00	1.00	6.73	15.0 - 40	10.26
6.4	4.05	0.95	6.97		
5.9	4.10	0.80	7.27	Urine remains clear orange	
5.0	4.21	0.60	7.71		
3.9	4.37	0.40	8.09	Urine turns cloudy now, brown	
3.7	4.45	0.10	8.35		
3.3	4.54	0.00	8.44		

Cultures VOC's now @ 40 ppb. Increase ventilation.

The two urine samples show some strong difference between color change and pH precipitation levels. We may also have two equilibration points on the second sample - take look at the curve.

There is a significant shift to the right of the second urine sample. It appears to have a much greater buffering capacity.

Sample #1 has an initial pH of 3.40
Sample #2 has an initial pH of 3.55

And yet it seemed as though sample #2 required a lot more acid (H_2SO_4) to bring it down to pH of 3.55. And yet sample #1 still starts at a lower pH.

This mean that the shift in sample #2 pH curve to the right demonstrates significant additional buffering within it, and thereby the ability to retain. There is no legitimacy in obtaining the two curves to match equilibrium volume points.

The crossing point of the initial pH of the two curves is @ ~ 3.3 ml of titrant. This mean that sample #1 took an additional 3.3 ml of titrant to produce similar initial pH conditions.

Also during titration, you noticed that sample #2, with an initial high pH of 6.4 demonstrated significant buffering capacity for a significant period, much longer than sample #1.

Notice the rise in sample #1 begins @ ~ 11.1 ml and sample #2 the rise begins @ ~ 16.8 ml
$\Delta = 5.7$ ml - significantly different.

Now let's determine the equivalence point & pKa of sample #2.

Ok, I have refined the curve comparison to get the best data / curve. Sample #1 titration in Oct 15 w/ Ka determined as 1.94E-4. (pKa = 3.67)
The ~~zero~~ 3.5 pH common ~~quality~~ point is actually @ ~ ~~11.2~~ 6.7 ml
Very significant to exclude this.

This means that we can subtract 6.7 ml from base curve volume & establish a common starting point.
This works well.

This shows that when Sample #2 is @ pH = 3.65 Sample #1 is @ 3.55. They therefore have essentially the same starting point.

Some important observations now take place with the two curves equated to one another.

Sample #1 starts the curve incline @ ~ 1 hr.
Sample #2 " ~ 1½ hr

These indeed demonstrates the sample #2 has a much greater buffering capacity.

Secondly, sample #1 has a very significantly steeper ascent than #2.

This also indicates that sample #2 has a greater buffering capacity

As ever if both samples were to contain formic acid, sample #2 would manage that titration for better than sample #1 would.

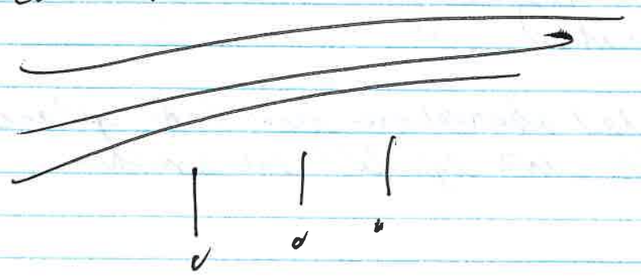
Proper Facts Here
in addition to Peter's Discussion

* Buffering capacity of the blood and urine they've managements

* shown itself to be an extremely important mitigation

* factor for TSP damage control.

This is exactly what was suspected to be the
Case.



Now let's determine the equivalence point and Ka & pKa for sample #2.

Or a reiterate, it is interesting that sample #1 shows the possibility of two equivalence points? Ideas Curious, w/out a doubt.
I am now looking @ smoothed curve.

I estimate the equivalence point of sample #2 @ X = ~~19.4 ml~~ 19.3
w/ pH of ~~7.50~~ 7.45

½ eq volume = 9.65 ml pH = 3.80
ie pKa = 3.80
$10^{-3.80}$ = 1.58 E-4 Once again, not by alot
in the range. Formic acid is 1.78E-4.

The evidence that both samples do contain formic acid however sample #2 has a ~~buffer~~ buffering greater buffering capacity and Chattle acid-base management of formic acid present.

There is now to be coupled w/ blood observation.

In the future, if you standardize acidity titration to a pH of 3.6 you will be at a common point for any samples evaluated.

Is there any region w/ acidity w/ H_2SO_4 vs HCl?
I do not think so since both are strong acids and would dissociate completely.

Today we went to look @ blood and titrate
C with acid as a independent reference.

The additional inflection point in the titration curve
is also of much interest.

Also notice that the color of sample #2, after
settling has now turned black from the
chocolate brown to a more expected
orange color.

Now for coupled observations of blood.

Sample #1 is my blood and my urine. The
blood looks as good as I have ever seen it
The free standing cells, of which are of course to find,
are free of CBC.

Everything says that the enzyme strategy of
interplay w/ protein development is working
extraordinarily well. Some grouped cells
do show CBC existence but overall it is
dramatically decreased along with vascular
& coagulation symptoms. Free standing
cells are easy to find. It looks to be
a marvelous accomplishment in physiology
keeping w/ a sensible strategy of disruption
on the front end of the Circle of metabolism.

Sample #2 (female, 75 yrs old, healthy diet) also look quite decent as far as lack of symptoms, save CDS presence & normal cell globules.

There is however, significant roulleaux in the sample. It is difficult (very) to find free standing cells. Considering that no strong enzyme regimen is in place, most blood and buffering capabilities in the urine are generally quite favorable.

If the roulleaux was reduced or eliminated it would show itself to be in favorable condition.

Both individuals are away from strong ELF frequency EMF fields in general however wireless internet is available. No cell towers are in range here.

The result of the recent work over the last couple of months is highly favorable.

The enzyme regimen will continue. Ultrasound will continue on skin breakout especially now in the lower leg. Existing polymer formation and formic acid encryptions through the skin will require continued gradual reduction. This is a slow and painful process. Years of effort have been involved but hopefully the process of removal is under acceleration due to recent knowledge acquired. Existing water polymer formation (eg, any clotting) remains highly troublesome and dangerous.

One primary focus now is to learn how to increase the buffering capacity of both the blood and the urine. In addition, there is a desire to raise the general pH of the urine toward the upper range (5 - this range). Current pH ~6.0. Sample #2 shows a pH of 6.4 and looks very favorable from the buffering capacity point of view.

There is no desire to change the pH of blood, or else the individual will be deaf. Increasing the buffering capacity of the blood, however, is desirable.

pH Digestion, pancreas function and capability are expected to be very important here. Bicarbonate ion & citrate ion also anticipated to be important. Research into bicarbonate - citrate buffer. Historical observation of citrate effect upon culture will come into play. The body's buffering system is required to be understood. Observation of increased bicarbonate - citrate ion will be made w.r.t. pH urine changes.

H_2CO_3 Carbonic Acid (weak acid) $\rightleftharpoons$ H^+ HCO_3^- bicarbonate ion (weak base)

One definition of a weak base is that it can bind or absorb to hydrogen ions. In both the definition the bicarbonate ion is studied a weak base.

(carbonate is CO_3^{-2}

Bicarbonate HCO_3^-

bi carbonate does not mean "two" carbonates, it means an a Carbonate ion has been bound to a hydrogen (weak base)

Carbonate CO_3^{-2} ⇔ Bi Carbonate HCO_3^-

Not entirely true to say that the term of "two carbonate" but there is not it. It means the Carbonate ion CO_3^{-2} is now bound to a hydrogen HCO_3^-!
So that soda is going to be $Na + HCO_3^- \rightarrow NaHCO_3$

Now you should understand why sodium bi carbonate is a weak base, it is because the bi carbonate ion can bind to hydrogen atoms

$HCO_3^- + H^+ \rightleftharpoons H_2CO_3$
(bicarbonate ion) (weak base) hydrogen ion carbonic acid (a weak acid)

This IS a buffer system.

a weak acid - conjugate base is the very essence of a buffer.
Now, notice that has nothing to do w/ citrate (how got a citrate -

The Carbonate ion is CO_3^{2-}

so if you have HCO_3^- " you see why it is -1 and understand that the bi" refers to a combination with hydrogen, not two. Bicarbonate DOES not MEAN TWO CARBONATES. It means that a carbonate ion is bound to H+, this is not intuitive.

A base "binds" a goins with an H^+

There is where the proton donor/acceptor definition of an acid come from. Now you know why ya need it. Not everything that acts as a base has an OH^- as a part of it.

And the Carbonate ion CO_3^{2-} is an excellent example. It is favorable to bind w/ H^+, and become HCO_3^- and is then, by convention, called a "bi Carbonate".
The Carbonate ion therefore should be acting a a weak base also.
But furthermore
HCO_3^- is also conducive to bonding w/ H^+ and therefore it should act as a weak base also and will therefore be prone to forming Carbonic acid, which is H_2CO_3.

The is very interesting. It generally extends your understanding of acid-base definitions in a very practical and real fashion. Not everything has OH^- bound!

Anything that is favorable to H^+ bonding helps in the understanding of an acid entering into the picture, and thus altering the stoichiometry of acid-base Chemistry to be extended much further to accomodate real life needs, i.e, the

UNDERSTANDING OF BUFFERS!

Oct 18 2023

I have increased the rate and volume of culture production even further - the 6 quart incubator can serve well both as a production source as well as for evaporation and concentration. It will probably take a full week for the culture to develop sufficiently and another week to condense. It is therefore a longer term affair but final production should be @ the quart level and a concentrated form @ the half-pint level.

We want to

1. Titrate an acid such as Citric acid
2. Investigate a mixed acid
3. Continue w/ buffer study, esp citrate - bicarbonate
4. I have some very good chem simulator software now:
   1. Chemlab - Good instructions
   2. Real X
   3. ChemCollective
   4. E-LabEz - look quite amazing

   } look quite capable
5. I also have some useful documents & study on buffers
6. NIR Can now be fully processed in lab now after satellite data collection.
7. Head towards electrochemical capability in lab.
8. Monitor urine pH w/ citrate - bicarbonate trial Titration monitoring.
9. MathCad now in place.

Superb - urine pH up to 6.23 after first citrate - bicarbonate trial.

Prepare additional NaOH-KOH titrant.
Existing mass of titrant + vessel = 376 gms

Use a ratio of 3 parts H_2O to 1 part base
4 parts total.
Add 50 gms NaOH-KOH = 426 gms
Add 150 gms H_2O = 576 gms
Total added 200 gms NaOH-KOH titrant

Citric acid is $C_6H_8O_7$ We know that conjugate base is $C_6H_7O_7^-$

MW = 192.125 gms/mol
1.0
a ~~0.1M~~ solution for 220 ml require 42.3 gms
Therefore a
~~0.01M~~ solution for 220 ml = 4.23 gms in 220 ml
0.1M

The mass of the total solution should be 221.50 gms
Mass of weight boat = 2.70 gms

2.70 gms + 4.23 gms = 6.93 gms

Water = 101.91 gms → 220 − 101.91 = 118.09 gms
101.53 → 118.09 − 101.53 = 16.56 gms
Done. 0.1M Citric Acid Solution created ~~220~~ 220 ml.

Because we have a known concentration now, we should be able to calibrate the NaOH-KOH solution now. (Maybe)

Either way, for now let's titrate the 0.1 M citric acid analyte w/ the $3H_2O$, 1 NaOH-KOH analyte

I am condensing the starter culture (starch for the foreseeable future now) and VOCs are present @ 20 ppb.

I am using 50 ml of 0.1 M Citric Acid
pH is measuring @ 4.42

Citric acid has 3 pkas 3.13, 4.76, 6.40

So Citric acid is a triprotic acid. That affects things quite a bit. Citric acid pH calculation is not like any normal agent. An initial theoretical computation of 0.1M citric acid is ~2.06
We are not even close. Let's check our meter calibration.

I am going to use 2 point pH calibration.
You need to get some pH calibration solution.

You now read a pH of 2.04. That is amazingly close. Article even stated when pH was a little high in theory. That shows the importance of full two point calibration. It also says that your pH measurements then you have been too high. That may end up screwing with affecting the culture growth treatments that resumes.

VOCs now up to 50 ppb. Increase ventilation.

The also means that we will repeat the titrations for formic acid, urine titration etc to see how close it differs.

Now we know that we do not need to acidify the citric acid solution, this is better. $\Delta t = \sim 30sec$

Burette Volume	pH
19.5	1.99
19.0	2.18
18.8	2.36
18.6	2.56
18.3	2.74
18.1	2.92
17.9	3.09
17.7	3.29
17.4	3.52
17.1	3.73
16.8	3.98
16.45	4.21
16.2	4.44
15.8	4.70
15.25	5.07
14.9	5.40
14.5	5.69
14.0	6.08
13.8	6.57
13.7	7.95
13.3	11.77
13.1	12.22
12.8	12.50

$\frac{1}{2}$ eq = $(19.5 - 13.5)/2 = 3ml$

$19.5ml - 3.0ml = 16.5ml$

$pKa = 4.18$

$10^{-4.18} = 6.61E-5$

Now the closest pKa stated in
3.13
4.18? $\bar{x} = 3.95$
4.76
6.40

Check: $(19.5 - 13.5) = 6ml$
$\frac{1}{2} = 3.0ml$
$19.5 - 3 = 16.5ml$

$\bar{x} = 13.5ml$ pH = 9.86 Eq. Pt

Now if we stated that the two pKa's 3.13 & 4.76 are very close together. You suggest that it may be very difficult to distinguish them from one another.

Note that pH jumped close to 4 pts w/in a range of 0.4 mL. There is an average 0.1 mL per pH unit. You do indeed not have the level of resolution in your method as to explain how it would pick up a single pKa somewhat as an average between pK_{a_1} & pK_{a_2}. As ultimately the does make a lot of sense, all OK.

Now let's measure a urine sample pH.

The above example shows you to be alert that multiple pKa's can be tricky to distinguish. Keep an eye on this for multi-protic acids.

With calibrated pH meter, my urine sample now measures ~5.5. Before trial, yesterday it would have been closer to 5.0 (too low)

Now the calibration is really into question the formic acid determination. The calibration suggests the pKa of the TSP acid might be closer to ~2.7 or so. This could change the picture considerably.

Ka might then be on the order of $10^{-2.7} = 2.00E-3$

Phosphoric

~~Phosphorous~~ acid may come back into the picture

Ka = 7.1E-3

* * *

Phosphoric Acid Returns a Candidate.

Guess what? From the synoptic chart, nothing is particularly close except for phosphoric acid.

H_3PO_4 $H_2PO_4^-$ Dihydrogen phosphate ion.

It is the first pKa that apparently is by far the most important.

We may well have come back full circle to the earlier research.

This is obviously potentially very important and in many ways quite consistent w/ historical precedent.

Phosphoric acid is considerably stronger than formic acid.

This would have the pH of our acid @ about 4.5 which is what we recall from before. To have a pK of ~2.7 and a pH of ~4.5 our concentration level would be on the order of .0021 M

Very very sensible.

Caution: Fe(III) has a pk of 2.17 "Hydrated Metal Ion"

Ok, this is a big deal. We are almost certainly now relieved to be dealing w/ phosphoric acid, not formic acid. The is completely consistent now w/ previous work.

The pH of the TSP, even plus prior to concentration is now at 4.58.

This means the pk is now expected to titrate out @ ~2.7 and the pH of our acid well be ~4.5 (what we now know it is) and a concentration level on the order of .002M.

Titrate as soon as possible. See if you can get away w/ not acidifying the TSP.

Phosphoric ACID $pKa_1 = 2.14$
$pKa_2 = 7.20$
$pKa_3 = 12.37$

No, let's acidify

THIS IS VERY FRESH TSP CULTURE.

Vol	pH	Vol	pH
19.75	1.57	16.6	7.87
19.5	1.58	16.3	8.70
19.2	1.61	16.1	9.05
19.0	1.81	15.75	9.39
18.7	2.91	15.3	9.71
STIRRING CRITICAL		14.8	10.05
LAYERED PH		14.2	10.81
18.4	3.48	13.7	12.05
18.2	4.04	13.2	12.43
18.0	4.65	12.8	
17.75	5.28		
17.6	5.96		
17.2	6.40		
17.0	6.84		
16.8	6.90		

PRECIP @ 6.8 - Sharp flattening

Because this TSP has not yet been concentrated, your titrant could be ½ as strong.

Phosphoric Acid Is Confirmed

1st Pt Vol = 1.63
1.63/2 = .815
~~pH = 2.08~~
match ∴ $pKa_1 = 2.08$

2nd Pt 5.8 ml
5.8/2 = ~~2.9 ml~~
pH = 7.0 a_2 $pK = 7.0$
match

Oct 19 2023

Heterotrophs on the latest TSP culture (large volume)

1. PH = 4.72
2. PPT = 62 (refractometer)

Increasingly I have suppositions that the TSP in native form (filtered, no precip, potentially no heat or evaporation as well)

may have more than one acid present. This is due to the complexity of the titration curve yesterday even prior to only locating a Concentration of just — only patterning was applied.

Although phosphoric acid now becomes a leady candidate upon pH meter calibration, the complexity of the curve indicates other factors present.
Strategize to assess the inclusion

1. The effects of heat
2. The effects of precipitation and decantery.
3. Be aware that precipitation is now likely to occur @ pH ~5.5, not 6.5 due to pH meter Calibration
4. Study of mixed acids in titration
5. Chromatography may come into play here

Urine ~ 1 hr after sodium bicarbonate - calcium citrate is pH 6.94 - excellent.

Another thing that has changed is that the concentration period of the TSP no longer takes as long as the TSP with the incubator. The incubator is more efficient w/ large scale concentration / evaporation. It has the effect of lightening the color/tint of the TSP.

Heating the TSP during evaporation @ 130-135° does oxidize it and turn it to a darker color

What we are doing next is bringing the TSP up to pH 7.4 w/ the calibrated pH meter. I will then decant and titrate.

I will also start cultures both
1. precipitated and decanted & variations there of.

Oct 20 2023

A culture reset is in place. This is due to the pH meter calibration discovered. There is a world of difference taky place in the range of 4.5 to 7.5 and I now start zeroing in on this difference and calibration of the meter is essential.

Also a larger volume of cultures is now being made over ~ 1 week interval [Inoculation and testing when the lab is empty now as the Montana winter is headed in...]

The cultures will all include starch for the time being.

Remaining work now is:
1. Acid(s) identification
2. Increased mobility & Good pH Control of Cultures.
3. Buffer development & analysis, and incorporation into culture work.
4. Mitigation monitoring - blood & urine jointly required.

It is interesting how quickly a crystal surface layer is formed on the first culture that has barely been started. Not even in the petri dish yet. pH was set to 7.4 on 10/19. No decant.

It will be not be practical to monitor pH in the petri dishes - insufficient volume. Possible paper route for coarse monitoring. Intent to maintain an independent sufficient volume for general monitoring

Some rather extraordinary culture macro development on the surface layer of TSP12 A-B w/ starch cultures. The layer does act like a polymer layer. The macro appearance is taking on some similarity in appearance to that w/in the paper "Enzyme Induced Disease ?" written many years ago. Macro level filament appearance.

Hazarding a guess under the scope, it appears to consist of a CDB-polymer "mat". It has some similarity to the apparent polymerization of the TSP-alcohol mixture test. It is a fine grain uniform and a dense layer. The cores from the TSP12 MS-B culture when pH was set to an apparent "7.4" - we know now it was most likely actually closer to 6.4. Therefore more acidic than presumed, but still far above the native TSP pH of ~ 4.5.

Still in the process of concentrating the next TSP batch culture trial density and pH monitoring will begin.

The first thing you learn w/ calibrated pH culture monitoring is that along with the rapid surface crystal development on TSP 50 set initially to pH 7.4 is that the pH drops to 6.9 in the 24 hr period.

The reason I can't do it show that acid production continues in a steady fashion. pH maintenance of the culture is the key to growing a live very important & when you want to maintain a biological pH of 7.4. I need to see what I can do to get a pH ready in a petri dish w/out disturbing the structure of the culture.

W/ careful control, I am able to get a pH ready w/ the meter in the petri dish. Adjusting pH, however, will be very difficult as

? 1. Any stirring mechanism will destroy the developed structure of the culture.

? 2. Stirring is required to get an accurate and even medium.

One thing that we could do is calibrate a larger volume reference vial from a differential pH method.

The reference vial mass is 11.08 gms
Reference + reference volume mass is 46.80 gms
Δ= 35.72 gms

I am going to alter and adjust the pH of the reference volume.

pH of TSP 50 petri dish is 6.98

Now setup reference vial for differential pH adjustment w/ stirring possible:

Next we need to decide if we use the undiluted NaOH-KOH or the 3 to 1 dilute NaOH-KOH.

The dilute NaOH-KOH will be easier to use. OK, will do, This should work.

Initial pH = 6.92
+50 ul +10 ul +20 ul +10 ul brings pH up to 7.4

Therefore 90
~~80~~ ul 3 to 1 NaOH-KOH = ΔpH of 0.48 =
35.72 ml (gms)

~~So ΔpH of +0.1 = 0.1 / 0.48 =~~

Let's determine mass in petri dish.
Petri dish mass w/ Lid = 13.86 gms
TSP 50 Petri Culture Mass = 38.78 Δ = 24.92 gms (ml)

We now have enough information to adjust the pH of Culture TSP 50 "remotely". Every culture will probably need to be calibrated as the fashion as the concentration of the TSP batches will vary over time along w/ combined pH change over time

90 ul / 35.72 ml = ΔpH 0.48

X ul / 24.92 ml = ΔpH of (7.4 - 6.98) = ΔpH of 0.42

Therefore, divide equations:

$$\frac{90\,ul}{35.72\,ml} \cdot \frac{24.92\,ml}{X\,ul} = \frac{\Delta .48\,pH}{\Delta .42\,pH}$$

$$\frac{1}{X\,ul} = \frac{.48\,pH \cdot 35.72\,ml}{90\,ul \cdot 24.92\,ml} \cdot \frac{\Delta .48\,pH}{\Delta .42\,pH}$$

$$\frac{1}{X\,ul} = \frac{1.433\,(.48\,pH)^2}{.42\,pH \quad 90\,ul}$$

$$X\,ul = \frac{.42\,pH \cdot 90\,ul}{1.433\,(.48\,pH)^2} = \frac{37.80\,ul}{1.433\,(.48)\,pH} = \frac{54.96\,ul}{pH}$$

Now a reference

$$\frac{90\,ul}{35.72\,ml} \rightarrow \Delta pH\ .48$$

If we want to change by $\Delta pH = .1$ then $\frac{1}{.48} = .21$
and $.21\,(90\,ul) = 18.9 \cong 19\,ul$

So to change control solution a ΔpH of $+.1$ we would add
19 ul NaOH-KOH (3 to 1) per 35.72 ml

Since our culture is 24.92 ml we would add
$\frac{24.92}{35.72}(19\,ul) = .70(19\,ul) = 13\frac{2}{3}\,ul$ per $+.1\,\Delta pH$

Since our ΔpH is $7.4 - 6.98 = .42$
We need to add to TSP50: $13.2\,ul\,(4.2) = \underline{55\,ul}$
to bring pH up to 7.4.

The method will take some work and time to maintain, but it is worth it. Tight pH control and monitory of culture becomes paramount. Temperature will take a little picture afterwards.

The tool gave a lot of work to set up a single culture but now you know what you have. 18 Different
* Growth forms w/out even trying in the reason why you need this.

This, and all cultures under the strict control, will be very interesting to monitor. In theory you should
* be able to minimally disturb but still adjust the pH of each culture. Undisturbed surface layer development has shown itself to be extremely important,
* eg Crystal biology & synth blood development sequence then.

Formic acid will come from Dida Diesel email 10/17 0920.

Our next move is to empty the solution and use decanted precipitated TSP for the next titration trial. The steps will be then to:

1. Precipitate
2. Decant
3. Acidify
4. Titrate

Start w/ pH measurement of natural TSP, no precip.
pH = 4.76

Precipitated to pH 8.1

It looks like it will take 3-4 hrs for the precipitate to settle out - you need to account for that in the planning.

Therefore you need to produce more of this.

Be aware that

1. TSP 50 (individual culture)
2. TSP 60 Three

are not heated or condensed nearly as long as what follows ie 24 hr heat vs 48 hr heat. There are other factors that may affect outcome.

Stick right now is to produce enough material to decant to begin a titration w/ simplified solution.

We have enough material from TSP 50 to decant and begin a titration. Designate as TSP 52

* ~~Here is the next major discovery~~ VOID. Very important.
~~The decanted TSP~~ has a pH of 8.07
~~There is another phenomenal.~~
~~The tell~~ is that the acid is in the precipitate!
~~not to~~ decanted by mistake.

VOID

The above is not true. We precipitated TSP 50 to TSP 51 which was raised to pH 8.1. All is on as expected. TSP 52 has a pH of 8.1. Acidify for titration. Acidified w/ H_2SO_4 to pH of 1.22

TSP 52 Titration – Decanted

Burette Volume	pH	
19.75	1.18	Eq. = 8.02 Δ = 3.55 — pk's? 1.8? 10.2?
19.50	1.23	½(3.55) = 1.775
19.10	1.29	1.775 + 19.75 = 17.975
18.9	1.40	
18.5	1.53	
18.0	1.78	½ Eq = 1.815 = pka $Ka = 10^{-1.815} = 1.53E-2$
17.8	2.06	$pka_1 = 2.14$; $ka = 10^{-2.14} = 7.24E-3$
17.4	2.50	Phosphoric Acid
17.2	2.96	Nothing else is close again
17.0	3.45	
16.9	3.88	~~2nd Eq = 13.4~~ ; (19.75 − 13.4) = 6.35
16.8	4.35	~~VOID~~ 6.35/2 = 3.18 ml — 3.55 + 1.775 ml
16.6	5.19	19.75 − 3.18 = ~~16.6 ml~~ = 14.4 ml
16.4	6.65	pKa ≅ 5.19 → ~pka_2
* 16.3	8.02	actual pka_2 = 7.20 ≅ 10.2
16.2	8.56	
16.0	9.04	
15.7	9.34	
15.35	9.55	
15.0	9.78	
14.7	10.06	
14.1	10.38	127
13.8	10.76	107
13.7	11.08	133
(13.4)	11.48	175 ←
13.2	11.83	125
13.0	12.08	
12.8	12.29	
12.4	12.49	

Now, plotting the titration curve presents a different picture. We now see a fairly Eq point @ ~ With derivative analysis, a definite Eq. Pt @ X = 3.3 ml

½ (3.3) = 1.65 ml

19.75 − 1.65 ml = 18.1 ml

pH = 1.73 = pka

$Ka = 10^{-1.73} = 1.86E-2$

Titration curve shows us that it is a weak acid as gradual slope, but primarily a strong weak acid since initial pH is down to 1.18 as defined w/ H_2SO_4

Ok, I think we need to be careful here.

Sulphuric acid has a K_a of 1.1E-2 w/ pka of 1.99

Phosphoric acid has a K_a of 6.9E-3 w/ pka of 2.16

Our K_a is estimated @ 1.8E-2 w/ pka of 1.73
Thus is actually close to sulfuric acid
I do not think that you can acidify w/ H_2SO_4 as it
will disturb the results.

We can prove this by titrating H_2SO_4.

Sulfuric acid & Phosphoric acid have pka values which
are far too close to one another. The fact that
the titration curve has characteristics of a weak acid
titration tells us that we probably have a mixture
here.

There a flaw in the method here.

Actually it is the sulfuric acid conjugate base
that has the pka of 1.99. HSO_4^-

H_2SO_4 has a pk of -3? Is that does not apply
or can have
Yes, strong acids do indeed have negative pka's!
pka of HCl is -7.

Substituents also affect the strength of an acid.

What are substituents of acids?

Oct 20 2023 (cont)

I see now how to get the 2nd pka of a diprotic acid, and for that matter, how to get the third of a triprotic.

Steps are

1. Find the first equivalence point (tangent rule) and get the volume that corresponds to it
2. Cut it in half.
3. Find the pH that corresponds to that volume — this is the pka. and $Ka = 10^{-pka}$.

4. Now for the second pka, double the volume of the eq point.
5. Now cut that in half (mean it's the same)
6. Add it to the volume corresponding to the first pka
7. Determine the pH corresponding to that volume. This is pKa_2.

Some principle for triprotic (?)

1. Triple the volume of the first eq
2. Cut that in half
3. Add it to the volume corresponding to the first pka
4. Determine the corresponding pH

] these ideas need to be verified.

So I see why I had it wrong.

Phosphoric Acid pka_1 2.16 pka_2 7.21 pka_3 12.32
Sulfuric Acid −3, 1.99
Iron(III) 2.17 (Hydrated Metal Ions have a pK!)
Formic Acid 3.75

How do you know if you have a hydrated metal ion? or an acid?

A very important topic here - you have seen the hyphae to some degree.

Iron(III) oxide-hydroxide

Our acidity here could well be resulting from hydrated III iron.

About as strong as acetic acid.
Color is a clear yellow, brown.
pH can be as low as 1.5 !

* Iron III oxyhydroxide precipitates from solution of IRON III salts @ pH between 6.5 - 8

* Then the oxyhydroxide can be obtained in the lab by reacting an iron salt, such as ferric chloride, ferric nitrate, with sodium hydroxide.

look these up. * The oxyhydroxide prepared from ferric chloride is usually the polymorph β (aka goethite) often in the form of thin needles.

* A lot of these is soundly familiar.

It looks like I am likely on to something here.

The acidic nature may be derived here from a role of iron, not an organic acid!

There is much more to what is happening than just needles.

* However looking for an organic acid may be a false chase.
BUT WE MAY WELL HAVE BOTH!

Do not discount the extreme skin irritation and discomfort akin to "stinging nettle, bee venom, etc.

The mean that formic acid remains on the table.

You may well have a combination of the Fe(III) oxy hydroxide hydrated complex and an additional acid within — thus also giving the impression of a diprotic acid, but it might be monoprotic fairly as well.

Settle the question of ordinary additional acidity into the TSP for titration — how might this distort the results? The conjugate base of H_2SO_4 has a pK in range also.

Many many factors are coming into play here in the effort to get a handle on the acidic nature of the TSP!

* Seek full volume range use of your titrant. To get the most information (high resolution titration available)

In the end, disturbing or disrupting the acidic nature of the TSP might be all that is needed. But ultimately it would be of need to identify the type and nature of acidic chemistry present.

Oct 21 2023

Urine pH measurement in AM, prior to Carbonate — citrate use: 5.29 quite low. Also not a surprise. A useful reference point. 5.40

Several supplies how come in:

1. Formic acid — a very interesting acid, as it is the simplest organic acid that exists
2. Ammonium sulfate
3. Potassium permanganate $KMnO_4$
4. Sodium acetate trihydrate
   $C_2H_3O_2Na \cdot 3H_2O$
5. Ammonium acetate
   $C_2H_7NO_2$
6. Washing soda sodium carbonate
7. Borax
8. Keyed cables

Many of these items fit in w/ Robert Johnson DIY Chemistry

We have a very interesting topic that arose last night that may explain much of what is being encountered. The role of Oxy Hydroxide Fe(III) is going to be studied investigated here. It acts as an acid and has a pKa just like an organic acid.

We know that iron is central to all that is going on here, and that may well also include and explain some acidity findings

In addition, the act'g acidifying a solution needs to be examined carefully and controls established.

Need to compare the HCl (mixture caption unfortunately) w/ H_2SO_4 impact and stay aware of the pKa values involved, esp pKa_2 of H_2SO_4!

Also the existence of the iron hydrate complex in urine are to be considered.

The precipitation of iron hydroxide also is to be looked at. The considerations of a protein - iron hydroxide complex being formed upon precipitation of the TSP is also to be ~~considered~~ investigated.

The HCl acid mixture looks favorable for trials (Zepp Acid Cleaner) 10 drops in ~65 ml of H_2O brings the pH down to ~~2.02~~ 2.00 and the solution renders clear in color (even though the commercial product is green). MSDS says: (Code R43710)

HCl 5-10%
Amines 1-3%
dodecyldimethylamine oxide 1-3%

Urine pH after ~1/2 hr of Carbonate-citrate injection: ~~5.75~~ 5.79

Left to get the titration into a spreadsheet to reduce data manipulation and transfer.

With low concentration of acid (even though pH is low) the blue titrant is fairly strong.

We need to dilute our base titrant. We actually need to different ratios. One for low concentration and one for high concentration. Our TSP is showing labels to be of moderate concentration

10 drops of 5-10% HCl in 65 ml of H_2O is actually a very low concentration even though the pH is very low.

Lets make up 3 solutions of NaOH-KOH
10 to 1
30 to 1
50 to 1

Our current solution is 3 to 1
Change this to 10 to 1.

Mass of glass vessel = 248 gms
Mass w/ 3 to 1 titrant = 443 gms. Δ = 195 gms (ml)
25% of this is KOH-NaOH, 75% is H_2O
.25(195) = 49 gms (ml). .75(195) = 146 gms (ml)
49 gms + 146 gms = 195 gms
Now we want a 10 to 1 ratio (total = 11 parts)
~~(1/11)(49 gms) = 49~~
(1/11) NaOH + (10/11) H_2O = 350 ml (gms)
~~for HCl~~ NaOH · 10 = H_2O

Easier $C_1V_1 = C_2V_2$
$.25\left(\frac{146\text{ml}}{195\text{ml}}\right) = C_2 \therefore .0909 \cdot V_2$ $V_2 = \frac{536}{\text{~~402~~}}$ ml
Mass of vessel = 87 gms 87 + 536 = 623 gms (ml)

Titrant Prep

Make 30 to 1 titrant: (31 parts total)

$\left(\frac{1}{31}\right) V_1 + \left(\frac{30}{31}\right) V_2 = 420\ ml$ (net)

$V_2 = 30V_1$ so

$\left(\frac{1}{31}\right) V_1 + \left(\frac{30}{31}\right)(30V_1) = 420\ ml$

$V_1\left(\frac{1}{31} + \frac{30^2}{31}\right) = 420$ $V_1 =$

$V_1(29.065) = 420$ $V_1 = 14.45\ ml$ $V_2 = 30(14.45) = 433.51$

$.032(14.45) + .968(433.51) =$

$.462 + 419.64 = 420.1$ gms (ml) OK

$V_1 + V_2$ therefore $= 14.45 + 433.51\ ml \cong 445$ gms

Now another way & thereby about the same

$V_1 + 30V_1 \cong 420\ ml$

This is simple $V_1(1+30) = 420$ $V_1 = \frac{420}{31} = 13.54\ ml$

$30(13.54) = 406.54$

$\Sigma = 420\ ml$

Mass of glass vessel = 46 gms

$46\text{gms} + 13.54\text{gms} = 59.54$ gms

Next Glass Vessel = 243 gms

$243\text{gms} + 420\text{gms} = 663$ gms brings total volume

Ok, now we have it. The fractional portion is highly important in more concentrated solutions.

Our last one is 50 to 1 ratio

$V_1 + 50V_1 = 420$ ml (there a good value for the pint jars)

$V_1(1+50) = 420$
$V_1 = 8.235$ ml

Glass vessel = 45.96 gms
45.96 + 0.235 gms = 54.2 gms

Glass Vessel = 242 gms + 420 = 662 gms total mass.

I have now prepared useful reference titrants at 10/1, 30/1 and 50/1 ratios

Let's learn how much volume is in the titrate tube to flush it out.

Urine pH ~ 2 due to ter Carbonate - citrate ingestion = ~~7.2~~ 7.1 major impact therefore.

Once a day would me smart:
Mass of residual Volume w/ 5ml in Burette
11.80 gms - 2.68 gms (mass of vessel) = 9.12 gms
- 5ml safety factor = 4.1 ml

~~to 4 mls of dose~~ to 5 ml of residual fluid in safety in the titrate tube

This is a reference titration:
Because we got [illegible] 5 ml of titrant through titration tube has been cleared. Anything above, eg 10 ml, includes a 5 ml safety factor, which is probably good practice.

These steps (calibration and reference) are very useful. Next titrate up 5 ml of 50/1 NaOH.

I have completed the titration. The results will be entered and saved as an spreadsheets for ease in process. I have a smoothed curve and a differentiated plot. The titration is very smooth.

In the case I have two degenerate equilibrium points.
One @ 4.85 ml ⇨ pH = 4.19
The other @ 6.05 ml ⇨ pH = 8.34

Now on pka,: 4.85/2 = 2.425 ml ⇨ pH ≅ 2.21

Assuming monoprotic acids:
6.05 ml/2 = 3.025 ml ⇨ pH ≅ 6.4 = pka,
3.025 + 2.425 = 5.45 ml ⇨ pH = 6.4 = pka$_2$

pka of HCl is -6.3
H_2SO_4 is -10
Another source says $pka_1 = -2$, $pka_2 = 1.99$

And they're the ones conjugate and difficult to interpret.

Sulfamic acid, even if it existed (not stated on MSDS) has a pKa of 0.995.
Aqueous solutions are also unstable. The should not apply.

Then we have wildly different reports for pKa, of H_2SO_4.

What the titration says is that we certainly have other compounds mixed in.

We need HCl prove to run the trial.

Can the vendor confirm pKa2 of H_2SO_4 as 1.99
& have no HCl and I need it.

OK, HCl is on order. Expected 10 days.
You will have to set by until then.

Therefore Formic acid is now our best reference acid we have. It is weak and monoprotic and my sample is fairly pure.

I honestly have no idea what is in the vials of HCl I have but it appears to have either two acids or a diprotic. We can tell if it is diprotic because of volume relationships

for our case we have our first Eq. pt @ 4.85 ml
Our second Eq pt would therefore be @ 9.7 ml
Our first pka, is @ 2.425 ml at a pH of 2.21 = pka1

Our second point, if diprotic or polyprotic is expected @
$\frac{9.7\text{ ml} + 2.425\text{ ml}}{2} = 4.85 + 2.425 = 7.275$

pH = 10.8

No wonder it is confusing. You cannot titrate mixed acids (like di acid) unless

1. the pka's are different by @ least 4-5 units
2. The acid concentration and amounts are close to equal

There are very tall order to fulfill. Mixtures are far more complicated. The means you need.

1. Pure acids
2. Separated acids if they are mixed.

Evidently but I know of no other relatively pure pure monoprotic acid + base beyond the recently acquired formic acid. Everything else is a mixture.

pKa = 3.75 Ok, this is what we use as a reference

The is a fairly strong acid. Suggest we use 30/1 titrant w/ ~ 5ml formic acid in ~ 30 ml H_2O
- Use ~10 drops in 45 ml Formic Acid

The is still too strong. Use 5 drops acid in ~ 40 ml and use ~10/1 titrant

The is a very good looking titration. The spreadsheet approach is very useful as:

1. You enter the data once
2. Smoothing takes place automatically
3. You see the graph develop w/ each titration point
4. You get the derivative immediately.

Eq in @ 4.65 ml
4.65/2 = 2.325 pH ≅ 3.55 = pka

vs theoretical 3.75. The is very good.
A very smooth monoprotic weak acid titration
No complications here.

The shows what can be done w/ a pure acid and single component.

Ok, then was excellent word to show that a method is in place for a relatively pure acid single acid involved.

& see that formic acid emits strong VOCs.
Then even though it is highly dilute
5 drops in ~ 40-60 ml of H_2O w/ NaOH added 10/1.

Then is a very balanced titration.

We have a good method in place. Semi automated.

pH of urine ~ 6-8 Use of sat. bicarbonate-citrate
Excretion = 6.45

Then means that at body had a significant effect
over the day period. We expect to acidify over
night then.

Calibrate pH meter Comparison & assess drift.

Oct 27 2023

The formic acid titration looks to be pretty much
text book quality. It is important that I do not get
how a pure monoprotic acid to work with - it
would help. But it is coming, hopefully.

On tap:
1. Titrate the final decant
2. How many acids, if any, are in the TSP?
3. Iron hydrate complex seems to be @ the ferment
4. What is the precipitate?
5. NIR
6 Electrochemistry in lab may now be important
7. TSP 50 Petri examination
8. RH blood urine pH, temperature collect
9. Buffer development
10. My own pH calibration solutions (?)

TSP 50 Petri shows an active enzyme layer. It is
a CDB mat w/ CDB enzyme crystals on occasion.
The cultures now ~ 3 days old. Now we
check on pH. Originally set @ 7.4. In 24 hrs
dropped to 6.9. We can measure pH directly
but we cannot stir it. We know how much
to adjust from the larger volume TSP 50 cultures

Calibrate pH meter before proceeding
pH Calibration was quite acceptable. Good.

Now measure pH of TSP 50 monitor.

Ok, the results are rather phenomenal. There is now the second adjustment w/in 3 days. The pH culture has dropped from the start pH of 7.4 down to 5.8. This shows that the cultured, even though heated for prolonged period at ~135° F, double cultured, precipitated from native pH of ~4.5, set and reset to pH 7.4,

nevertheless continues to produce an acidic state that seeks out the native form of the TSP.

This shows that there is a continual acid production taking place. The value and importance of buffering the early portion of digestion after the stomach is apparent. The location, eg the pancreatic function must be supported in a continuous basis.

Now we need to adjust the pH by proportional mass.

Mass of monitor vessel w/out lid: 8.26gms
Mass of monitor TSP 50 culture w/out lid: 43.38gms
Δ = 35.12gms
Now we are free to stir and adjust pH of TSP monitor culture. Keep close track of NaOH - KOH added.
I think it is best to use the 10/1 titrant.

Now, when I stirred TSP Monitor 50, the pH rose from 5.8 up to 6.1. This means that there is a buffering effect going on. It will be suspected therefore that the settled layer will have the lower pH.

Add: 50ml + 100 + 100 + 100 + 100 + 100 + 100
+ 100 + 100 + 100 + 100 + 100 + 100 + 30 + 50
+ 50 + 50 + 30 + 30 + 50 + 50 + 50

$\Sigma = 1640\,ml = 1.64\,ml.$

$\frac{1.64\,ml}{35.12\,gms} = \frac{x}{23.87\,gms}$ $\quad x = 1.12\,ml$

Petri dish w/ Lid = 13.86 gms
Cultrw TSP 50 w/ Lid = 37.73 gms $\quad \Delta = 23.87\,gms$

Add 1120 ml to TSP 50 Petri Culture to buy
pH up to 7.4. DO NOT STIR! mild agitation only.

The re-hydrate a dry sample. Can't change that
re-hydrated plant in the culture.

TSP 50 is an early forerunner sample of the latest
and now only tested culture. It appears that it
is a native format culture, not overly heated,
that was raised to biological pH of 7.4
It is an early native form TSP culture raised
and attempted to maintain biological pH. We
learn in the process that it becomes strongly
acidic, in a relatively fashion over time.

Next look @ two urea pH samples.
CEC prior to Carbonate - Citrate ingestion (AM) = 5.3
Low, not surprising.
Carbonate - Citrate ingestion @ 1300.

The next step is to investigate the TSP decant in more detail.

1. What water likely soon show up?
2. Is there an iron hydrate complex?
3. How can I titrate w/ wt HCl a monoprotic acid?
4. Is the protein by UV
5. What does NIR say?
6. What does electrochemistry say?
7. What culture variation would you like to set up?
8. How can you develop and apply a buffer that be value?
9. Can you incorporate a buffer into the culture?
   If so, what effect does it have.
10. Early leakage on the precipitate:
    Iron hydroxide Compound?
    Protein - Iron hydroxide Compound.
11. Discovered place of iron III hydrate complex.
12. Chromatography have an application here?
    Can it separate into parts the parts?
13. The master goal of all the abstracts?
14. MW determination?

If you demonstrate removal of the COB w/ in the blood, the primary objective is ~~reached~~ reached. The answers to the above may well help that process.

Let's start w/ an /on test
You actually only have 3 trials for the process.
1 Group of tests w/ 3 strips ~~test on each strips~~
and 3 tests on each strip.

There's not a whole lot to work with here. You need to think about how to best apply your tests.

I am guessing that we should start w/ the native TSP form, prior to precipitation a process of any kind.
It should therefore be stirred and mixed.

I will place ~~two~~ drops of the native TSP into the glass vial for each test. You must use distilled H_2O.
Concentration Level: 10 drops in ~ 3 ml distilled H_2O.

1st Test.

Carbonate : Ø
Cyanuric Acid : Possible ~ 10 mg/L
Sulfuretted Hydrogen: Ø

Cyanuric acid is a xenobiotic. A xenobiotic is a chemical compound foreign to a given biological system.

Lets put in 10 drops per vial

#2 Fluoride Ø
Chlorine Ø
Bromine Ø
Free Chlorine Possible ~ Ø.2 mg/L

Cyanuric Acid

10E-3 / 1000 = 1 / X X = 1 in 100,000 or 150ppm (CDC×10)
CDC pool limit 15ppm

is associated w/ free Chlorine & thiocyanates CYA is a metabolite of melamine

Results of Iron Testing for TSP – native form

#3 Zinc 0
Manganese Ø
Calcium 120 mg/L

#4 Lead 5 ppb

#5 Alkalinity Ø
Hardness 50 mg/L
pH <6

#6 Nitrate Possible ~5 mg/L
Nitrite Possible ~0.5 mg/L
Sulfate Ø
Sulfite Ø

#1 Copper Ø
Mercury Ø
Iron Possible 0.1 mg/L

The was a very fruitful set of tests to conduct. They established grounds for further targeted investigations. Concentrations of elements/ions anticipated to be low – however our sample was quite dilute, ~ 10 to 1.

Candidates are therefore:
* 1. Cyanuric Acid
* 2. Free Chlorine
*** 3. Calcium
* 4. Lead
** 5. "Hardness"
* 6. Nitrate
* 7. Nitrite
* 8. Iron

This is a good start for a list of candidates w/ probable cause

Notice that these must be in ionic form eg does not detect iron hydrated complex.

When you combine the with
1. UV - Protein testing
2. Amino acid - UV
3. NIR - organic
4. Acid base Chemistry - Titration
5. Electrochemistry - Inorganic
6. Microscopic examination
7. Culture observations - ~18 variations, growth, blood, Crystal testing, polymers
8. Chromatography separation?
9. MW determination/estimate?
10. Polymer analysis - alcohol?
11. Assay for testing.
12. Current influence - electromagnets

Then you can indeed get a handle on some of the constituents

The candidate knows of cyanuric acid and free chlorine he may intensely revisit before ever if ever a preliminary examination.

* Melamine is a synthetic plastic.
* Cyanuric acid is a metabolite of melamine.

How does H_2SO_4 affect affect the titration of an known weak monoprotic acid.

TSP 50 Petri is showing some fairly advanced growth after 3 days. Attempting to retain physiological pH @ 7.4 although it continually seeks acidic state. Present as

1. Fairly advanced crystals with step pulsed log. (Strep).
2. Lysth cells in menor numbers (apparently)
3. pinkish taint developing
4. ~~Poly~~ Polymer regions - extensive
5. All as always later.

A majority of what has developed all now in one small petri culture of 3 days age. I can disregard any remaining previous cultures now. All large glassware culture has now been replaced w/ a petri dish approach under stringent conditions.

I am not sure where VOC's are coming from. They have been present all day. There are no open bed cultures in place now. They are between 20-50 ppb. They become troublesome >50. A mystery as to their source...

The Sodium Carbonate (Washing Soda) Acid Test... (pH 11)

1. Native TSP (no precipitation) - a white precipitate forms. Some gas production (assume CO_2) early in reaction but it has dissipated shortly after.

2. TSP precipitated and decanted TSP used: No visible reaction. No gas production, no precipitate reaction.

Next up Sodium Bicarbonate Test (Baby Soda) pH 8.

Very interesting results here

1. TSP Native Format (no precipitation)
   Reaction: None visible. No precipitate
   No gas production (possible slight at beginning)
   ↑ NOTICE THIS (OCT 23, 2023)

2. TSP decanted:
   No visible reaction. No gas production.
   No precipitate reaction.

The suggests we are not dealing w/ a traditional acid (Ident of baking soda + vinegar) Expected CO_2 production not occurring except w/ sodium Carbonate & native TSP.

The hydrate entered as Fe(III) oxy hydroxide very acidic but not a traditional acid.

And what and why is the white precipitate formed with native TSP and sodium Carbonate but not with sodium bicarbonate?

The tests have good questions
1. Does sodium Carbonate change pH of Native TSP?
2. " " bi Carbonate " " "

Oct 23 2023

Here is what I am seeing further on the Carbonate test.
Under sufficient alkalinity in each trial, the extraction does change.

Baking Soda + NaOH Decanted TSP Trace of white Cloudiness No gas production	Baking Soda + NaOH Native TSP Moderate White Precip No Gas Production
Washing Soda + NaOH Decanted TSP Light production of White Precipitate No Gas Production	Washing Soda + NaOH Native TSP (and w/out add. NaOH) White Precipitate (Greatest production) Initial Brief Gas Production (CO_2?)

A picture is beginning to emerge here. The existence of an organic acid in high volume of the TSP is now more reminiscent as a consideration, but acids in general are not eliminated in any fashion. Recall recent indication of cyanuric acid's presence.

Increasingly we are drawn to a hydrated iron complex as being responsible for, likely responsible for the majority by the acidity present. The fact that the precipitate is white, however, is of interest.

Notice the white color of the precipitated TSP is the same as the white precipitate produced in the Carbonate test above.

The Carbonate was not the source of the precipitate, the alkalinity is.

The Carbonate test actually fails the "acid" test, but this does not mean there is not an acid as we learn from the nature of the hydrated iron complexes.

Question - do mineral acids pass the Carbonate test as an organic acid (eg vinegar) will? Try it with H_2SO_4.

Also examination of any crystal geometry may be helpful in identification of the precipitate. Chemix provide information here.

I am making some progress now. Yes, a mineral acid generates vigorous gas activity (CO_2) as does a organic acid like acetic acid (vinegar) does. The gas production is vigorous and brief, it is not sustained.

The means are brief gas observation w/ the TSP in native format is important. It means that we do have an organic acid present in the native form TSP, under conditions of sufficient alkalinity. Notice any and all gas production notes of work today and on Oct 22nd, 2023.

The organic acid presence will now be in addition to the acid nature of the iron hydrate Complex to ahead of us is:

1. What organic acid exists w/in the TSP?
   Cyanuric possible?
2. In depth study of the acid nature of the iron hydrated complex

---

3. Comparison of the TSP precipitate and the "Carbonate" trials precipitate (actually induced by alkalinity, not Carbonate) under the microscope to start.

4. The precipitate separation is therefore quite important. I am suspecting a protein-iron Complex.

5. Need to isolate the important differences that are showing up between the native TSP format and the decanted format.

6. All of the analytical methods available look like they are going to become very important.

7. What is the true nature of iron acidity? Maybe it will be the third definition of an acid that will be required.

8. Does the Carbonate-Citrate buffer apply to iron acidity?

9. Notice the pink color on TSP50 Petri. React w/ H_2O_2?

10. Very pressure in addition to angle on blood smear.

No VOCs this A.M.

There is very significant H_2O_2 reactivity w/ the TSP SD petri dish that has developed the peaked tongue.

Catalase / and/or hemoglobin reactivity is a strong and reasonable presumption here.

Interestingly enough, there are several prominent filaments (dark and red) readily visible in the small sample being recorded under the USB scope.

The reactivity has been recorded.

Catalase enzyme is a common enzyme that is found in all living beings that survive on oxygen and catalyzes the decomposition of H_2O_2, releasing water and oxygen.

The Kastle Meyer presumptive test uses PHENOLPHTALIN.

Check this

WHICH IS NOT! PHENOLPHTALEIN

THIS CLAIM IS CONTRADICTED

★ The test actually converts phenolphtalein (colorless) to phenolphtalein (pink)

Need phenolphtalein? Company on to other

Most sources are saying that phenolphthalein IS USED.

Video shows phenolphthalein being used.

I have order in place for (thru NOV 21)
1. Phenolphthalein
2. PH Buffer solution
3. Hemoglobin meter
4. Add. Hemoglobin test strips

Oxalic acid Comes Oct 25
HCl Oct 26-31

What we know is that we have Catalase. We can not prove hemoglobin yet.

There was no major difference in blood observation between the light and light pressure slide preparation.

My blood continues to show stabilization and improvement. It appear that one of the best goals is to have a highest density of pre-retarding cells as possible. After this coupled removal of the CDB from the cell interior. These traits continue to present more commonly now. It looks to be on the right track.

I will be studying the characteristics of a Carbonate-Citrate buffer.

Other buffers will also be explored and engineered upon.

There is a sodium Carbonate - sodium bicarbonate buffer ie Baking soda & washing soda.
Can't get much simpler than that. pH range, however 9.2 & 10.6. And most importantly washing soda is not edible. There is too Caustic.

Washing soda is dependent to use. (but not in small amounts)

Your shared term we going to be:
"alka seltzer buffer Chemistry"
plenty of references will appear under that
Here is the Chemistry:
Dissolved in Water:

$3NaHCO_3(aq) + H_3C_3H_5O_7(aq) \rightarrow Na_3C_3H_5O_7(aq) + 3CO_2(g) + 3H_2O$
sodium bicarbonate, citric acid, sodium citrate

the sodium citrate acts as an antacid.

So only a portion of the sodium bicarbonate is used

$Na_3C_3H_5O_7(aq) + HCl(aq) \rightarrow H_3C_3H_5O_7(aq) + NaCl(aq)$
sodium citrate, Hydrochloric acid, citric acid, salt

Excess sodium bicarbonate acts to neutralize base

if left over then

$NaHCO_3(aq) + NaOH(aq) \rightarrow Na_2CO_3(aq) + H_2O(aq)$
sodium bicarbonate, lye or base, sodium carbonate, water

Thus my goal is you only need the Citrate.
Adults have lower levels of stomach acid.
Calcium citrate is soluble in HCl.

Calcium citrate + HCl → Citric Acid + Soluble Calcium Ion

$Ca_3(C_6H_5O_7)_2 \cdot 4H_2O + 6HCl \rightarrow 2C_6H_5O_7^{3-} + 6H^+ + 3Ca^{+2} + 6Cl^- + 4H_2O$

$CaCO_3$ (insoluble) $+ 2HCl \rightarrow Ca^{2+} + 2Cl^- + H_2O + CO_2$

Calcium Carbonate + Hydrochloric acid → Calcium Ion + CO_2 + Chloride

Pancreas - bicarbonate ion?

Oct 24 2023

Today:

I wish to set up ~~two~~ 4 additional cultures. (Variants TSP Petri)

TSP53 1. 1 Native form TSP stored w/ pH up to 7.4 Incubated
TSP51 2. " " (Not Incubated) " w/ Blood Drop
TSP52 3. " " " " " w/ Blood Incubated
4. pH TSP 51, 52, 53 Monitor - Incubated

—

In addition, I want to look @ the structural form of the precipitated form.

Also want to look @ what has protein and what does not

—

Original pH of native TSP is 4.7

—

Check TSP of 50 Petri & TSP pH Monitor

TSP in the native format is highly acidic @ 4.7

How do you make an iron hydrate?

Monitoring pH of TSP 50: Surface Layer 6.2
TSP 50 Monitor: Bottom Settled Precipitate Layer 5.7

* The latter finding is important, as it shows that the settled layer (suspected iron hydrate protein complex) is responsible for the continued lowering of acidity of the TSP cultures, even after bringing up to biological pH. Hence the need for buffer development.

So we have a continuing decrease in pH of the TSP culture. Adjust to 7.4.

Mass of morita vessel w/out Lid: 8.24 gms
Mass of TSP morita vessel w/out Lid + TSP = 44.56 gms Δ = 36.32 gms

Shake and raise pH to ~7.4 (Mixed pH is 6.0)
100 ul + 100 ul + 25 ul 20 = $\frac{220 \text{ ul}}{36.32 \text{ gms}} = \frac{X}{23.11}$ X = 140 ul

Mass of petri vessel no lid = 7.18 gms
Mass of TSP 50 Petri Culture, no lid = 30.29 gms Δ = 23.11

Add 140 ul NaOH-KOH to TSP 50 Petri. Do not stir!! Swirl only.

You can now establish that mass of TSP 50 Petri is ~ $\frac{23.11 \text{ gms}}{36.32 \text{ gms}} = 64\%$ You can use this in future pH adjustments.

These culture variations and separations will be of interest to monitor. There is now also heightened interest in the nature of the precipitate, eg iron hydrate, acidity, protein existence, etc.

Incubated chamber has temp of ~130°F. Let's work toward ~100°F. Fluid mass should decrease temperature some.

Let's look @ TSP 50 Petri surface layer. Active development. H_2O_2 responsive. Pinkish tint. Blood now added to create a TSP 51.

OK, we have positively captured synth erythrocytic production w/ an FSP50 Pltr. Maintenance of biological pH @ ≈ 7.4. does there appear to be important. The cells are struggling w/ regular geometric development but they are clearly erythrocytes.

The surrounding polymer network is dense and obscures observation (below) beside the scope. This can be reduced by gently tilting the cover slip slowly from one edge and taking up the overflow with a tissue on the opposite side. It will increase clarity of the solution and aid observation.

"Sac" production also has been captured.

The culture process is therefore successful. Crystals and are abnormal and filaments non existent. There is a Polymer – COB – Erythrocytic culture @ this point. Surface layer development.

pH maintenance is obviously a challenge. Cause for buffer development.

OK, the precipitate is primarily a protein complex, as assessed by microscopic examination of the "Carbonate" testing. Precipitation was induced by alkanization.

So next steps are
1. Verify protein by UV and NMR if required
2. Verify gas production w/ "Carbonate" test on native FSP.

Then right now we are led to presumptions that
1. TSP may contain an acid above and they and the iron protein complex that precipitates out upon increased pH
2. Verify gas production w/ the TSP and Carbonate.
3. Verify protein complex w/ UV and even VIS for that matter.
4. Synth blood - human blood comparison paper.

On the TSP that has been precipitated there is TSPCO there, we have an emphasis upon an iron protein complex from the bottom settled layer. Synth cells are present. Many of them are oval shaped. They are of regular geometry even more so than the TSP (50-) petri culture. There are also crystals present, not highly developed.

What they say is that the TSP precipitate is not actually the same as the precipitate formed in the Carbonate test. They may have similarities and overlap but in general the the TSP precipitate is more complex w/ immature crystal development, clear protein development and synth cells that are of more regular geometry.

Treat them as separate entities for now.

They are indeed biconcave synth cells but slide needs to be flushed to view more clearly

So where are you headed now?

1. Variations in cultures under tight control
   e.g. pH, incubation, blood, precipitate, decant

2. Improvement and refinement of mixed cell production

3. Pump vs decant

4. pH – acidity analysis
   1. Acid present?
   2. Iron hydrate pH question
   3. Protein iron complex? – acid?

5. Paper on blood difference

6. Buffer integration w/in cultures

7. Two fundamental interference mechanisms established. Any others on the fundamental level?

8. ~~How~~ Deacidalkalyating iron hydrate complexes w/ OH approach?

9. Hemoglobin vs Catalase testing?

10. Monoprotic acidification & titration – HCl

11. Polymeric investigation – alcohol w/ TSP?

12. Chromatography? esp to decants?

13. Ba carbonate citrate and buffer monitory

sodium
One thing we see is that the carbonate precipitation method seems to produce a fairly opaque precipitate when microscopically viewed as a relatively homogenous pellet type.

Let's produce the agar and test w/ UV - protein reagent.

The method of precipitation w/ carbonate is (vs NaOH - KOH)

1. About ~~About 3 ml~~ + 20 drops of stirred TSP into 3-4 ml H_2O
2. Add about 1/8 tsp washing soda (sodium Carbonate). Precip will occur. Light gas production. Control then for acid detection (litmus). Centrifuge. Run through UV-VIS protein detection.

3. Before UV testing you must acidify again. 40 ml Use ~~1 drop~~ HCl in about 2 ml H_2O w/ decanted solid protein.

Now here are a couple of observations from the above:

1. H_2SO_4 definitely dissolved the precipitate and returned the solution perfectly clear.

TAKE BUT NULL VOID

2. A relatively large amount of gas was produced when the HCl was added.

The says we definitely have an acid present in the precipitate. The precipitate looks to be quite pure.

Carbonate Protein Precipitation/Purification from TSP.

~~I started the Carbonate test to see if I had any acid present.~~ VOID Well indeed I do. Steps are

1. Precipitate the protein first w sodium Carbonate what purify and precipitate protein.

2. Now if you add H_2SO_4 to it only prove that ya have an acid, namely H_2SO_4 via CO_2 production. Nothy learned here. You need CO_2 production from the Carbonate addition alone. In future too.

Running UV from 226 to 400 nm. Used 300 ml in Cuvette

We absolutely have UV absorbance with a strong defined peak @ 280 nm.

* Absolute protein detection established here.

* The value because by color and microscopic examination I think that we have a rather pure form of protein @ hand. We also know this protein form seem to be incriminated in various cultural developments.

Now one of your questions is if you can show that enzyme digest this particular protein.

Let's try a larger volume precipitation by the Carbonate method.

Done. ~ 30 ml solution.

Now lets try VIS protein detection 400-500 nm.

VIS Protein Detection w reagent also gives a positive result. Shift from Valley of 434 nm to 441 nm.

Protein existence is confirmed by both UV & VIS spectroscopy.

Carbonate 30 ml precipitation of TSP is now settling. This will produce a greater volume of purified precipitated TSP protein. It shows strong blue of purer form than that precipitated w/ H_2SO_4.

The has dynamite advantage. 97.8

Now is there an acid in the protein?
How would you show that in a solid form?

Oct 25 2023

The Carbonate method of precipitation may create a more pure form of a protein vs the NaOH - KOH method. Not sure yet. The passage of time, as had occurred w/ the TSP NaOH KOH precipitate may incur additional impurities and growth forms. I will need to compare them under the scope w/in the same time frame. In the time being, we can conclude that the Carbonate precipitation exists as a more pure form and this is useful.

—

We find that the precipitate protein layer is more acidic than the decant TSP. This was determined by the difference in pH between the decant upper layer and settled bottom precipitate layer. But both layers are acidic

The difference was Δ .5 pH units. Not large but noticeable and determinate.

Seems to me that you need to determine if you have a "conventional" acid (eg mineral or organic) in either layer (decant or precipitate) by the TSP. You can also see if you have one from the TSP as a whole.

There is close w/ the Carbonate test of Ca production, not precipitate production. Precipitate production by Carbonate is another matter - the Ca and does need to protein prep, but it can also lead to iron hydroxide precipitation.

Depends upon what you test, if still acidic but not from a "Conventional" acid, you will then look into metal complexes more closely.

Here are some ways to determine if an acid exists:
1. pH
2. CO_2 will be produced when added to a carbonate
3. With most metals, they will release hydrogen
4. They will neutralize (or lower the pH) of an alkali and will fizz at least in the process.

Now is this all true for an acid metal complex as well? How would you make an acid metal complex,[2] to test it.[2]

Aluminum + H_2SO_4 → Aluminum Sulfate + hydrogen

$Al + H_2SO_4 \rightarrow Al_2(SO_4)_3 + H_2$

+2 -2 -6 -6

That is quite interesting and works quite well. That will give me one +3 metal ion to work worth with.
Copper sulfate will give a +2. Ferrous sulfate a +2
Mg sulfate sulfate a +2

Ferric sulfate is to be made w/ a hot solution of ferrous sulfate + H_2SO_4 + H_2O_2. Not Made Out.

When I add sodium Carbonate to the native TSP, I see a small amount of gas produced. I think therefore that the mean we have an acid there.

We may therefore have three source of acidity w/in the TSP.

1. Carbonate - presumed CO_2 production w/in the native TSP indicates a "conventional acid", almost likely an inorganic acid. Cyanuric is one such candidate but that could likely be others.

2. We know that the proteins that are precipitated out by the ja w/ H_2SO_4, seem to be a significant source of acidity in the culture.

3. We suspect a major acidic contributor is coming from an iron hydrate complex (III).

—

So ja Carbonate has 3 functions ja is:

1. It produces CO_2 in the presence of an acid
2. It is expected to produce iron hydroxide, if it exists, we do not see that yet.
3. It is precipitating out a protein from the TSP, in a manner that seems similar to adding NaOH-HCO_4 but it seems as though the protein product is more pure than that precipitated of H_2SO_4.

—

It could be quite a challenge to count out these sources of acidity. Titration is also showing much difficulty when you suspect a mixture

Now we also see that not just acids have a pKa (a pK anyway), as amino acids also have a pKa and even hydrates also have a pK.

Therefore to identify what you actually have is almost certain to be very challenging.

It certainly would be of interest to know what type of acid is causing the stable low pH of ~4.5

1. Mineral acid?
2. Organic acid?
3. Amino acid?
4. Metal hydrate complex?

You see, there are many possibilities here.

We know that a mineral acid and an organic acid react w/carbonate to produce CO_2. What about the other two? eg amino acid, metal hydrate complex.

Let's try an amino acid. Approx 1/3 of the tablet is NAC! Is NAC water soluble? Yes. This helps.

All H+ donors are acids, but not all acids are H+ donors.

Transition metal complexes (coordinate or compounds & organometallic compounds) are essentially Lewis acid-base complexes!

Ok, there are metal complexes are in the last acid category - not as common.

1. Arrhenius H^+ Concentration

2. Bronsted-Lowry does not require water
   & ammonia
   - sometimes applies when it

3. Lewis - does not ~~need to~~ Contain hydrogen
   (& Boric acid does contain hydrogen but
   it is still a Lewis acid).

How did the acid definition evolve,

1. Arrhenius was based upon chemistry things in H_2O.

2. Bronsted Lowry is based upon how things react with
   one another, not how they react with water.

Classic Case

$$HNO_3 + NH_3 \rightarrow NO_3^- + NH_4^+$$

Notice no H^+

Nitric Acid — ammonia (a gas) — a nitrate ion — an ammonium ion

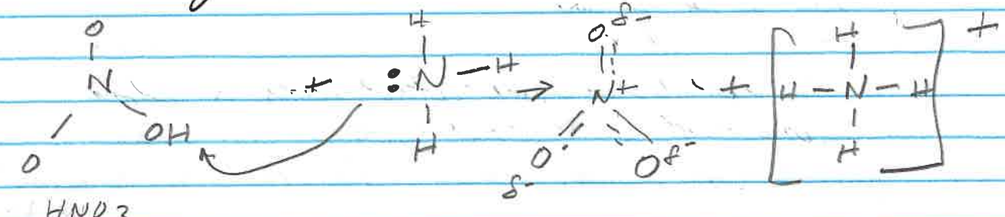
Notice no OH^-

Ammonia as buy is NH_3 (gas) + water (H_2O) → NH_4OH (unbalanced)
It is actually ammonia hydroxide

There are many times when water is not involved in the reaction. There is entropy factor. But hydrogen should be.

3. Lewis acid.

Even hydrogen atoms not need to be involved.
Lewis definition has nothing to do w/ protons.



HNO3 (Nitric Acid) — Ammonia — Nitrate — Ammonium

ie $HNO_3 + NH_3 \rightarrow NO_3^- + NH_4^+$

The same reaction as before but viewed in terms of electron transfer, not specifically hydrogen proton transfer.

Oct 26 2023

I want to learn about Lewis acid & base may well be involved in the TSP. Lewis acids and carbonates chemistry are intricately linked, so you have some study cut out for you.

I also want to run some control tests on alpha hydroxy acid & N acetyl cysteine. Do these generate CO_2 w/ carbonate? Is there any reaction?

How do you make a metal hydrate example?

Keep plugging away on how you will determine the nature of the acid(s) in the TSP. Determine between what is in the precipitate and the decant. It also raises the question of how you determine the pH of a solid. If you dissolve the solid in acid it sort of defeats the point unless you wanted in a different form, and that could make sense.

i.e. pH of a mixed solution.

Look @ cultures and pH monitoring

A very curious gain on TSP50 Petri.
Synth cells are not in large number but they are present. What is curious is that in a sectioning slide they are more abundant, but they are aligned in rows.

There is most curious. I need to retrieve magnets I should have stored.

Although not higher number, the cells do look to be generally circular and smooth globularly.

The pHy TSP50 petri has dropped once again. Now @ 6.72 from 7.4. Raise the monitor pH Container & adjust TSP50 Petri.

+60ul −30ul (H_2SO_4) +10 = Δ+40ul
+40 · mass ratio of .64 = 26ul NaOH-KOH to add
Done.

—

TSP51 Blood Added, no incubation, pH initially set of 7.4 on 10/24/23. No pH adjustments since. In the culture what distinguishes it is that some of the synth cells are CDB enfused (somewhat grouped possibly affected by human blood proximity). Other synth cells are formed normally. All synth cells seem to be ~4 um in diameter.

OK, have learned something important from TSP51.
The cultured CDB is actively destroying the introduced human erythrocytes. Well documented under the scope and a paper observer to be written

—

~~Also TSP52 is very important.~~ VOID. TSP52 Human blood cells appear intact. At this point it can not be stated that there are synth cells as human blood was introduced into the culture. The erythrocytes remain in good form within an incubated TSP after 48 hrs w/ pH ~ 7.4 — But here is something interesting

The pH Monitor has dropped in 48 hrs from 7.4 to 5.8!
Even the TSP51 Petri w/ introduced blood will have done this!
Both CDB destruction and acidity is important here now.

To the TSP 51, 52, 53 pH Monitor vessels we added 100 ml +30

Notice there is similar to what happened w/ TSP 50 Petri, even though it has not been incubated. I had a similar sharp drop in pH twice there over a 7 day period. But notice TSP 51 today only dropped * Δ pH = 0.7 and also only required 40 ml NaOH to bring up to biological pH of 7.4.

There is versus 130 ml now today of the pH 51, 52, 53 monitor. This indicates that pH eventually began to stabilize but it can take a while or more.

Now the many on TSP 52 (w/ blood, incubated) and TSP 53 (no blood added, incubated) is about the same as the TSP 51, 52, 53 monitor vessels. Therefore after observation of TSP 52 & 53 are completed today & will add 130 ml NaOH KOH to each to bring up the pH to an estimated 7.4 in both cultures.

Now, the sharp drop in pH on TSP 52 calls into question if the cells might be of synth nature & not tissue the pH perhaps may be no longer sustainable for erythrocyte maintenance as it is being absorbed.

The only TSP53 will now be valuable to observe as it has blood introduced onto it.

—

Here is what we see. TSP53 does have synth cells within it. They are identical to the cells seen in TSP52.
This tells us that the cells seen in TSP52 are also almost certainly to be synth cells, even though TSP52 had some human blood introduced onto it, and TSP53 did not.

It also tells us that incubation along w/ pH strict control is almost certainly essential to the process as the cells in both cases are in many cases reaching normal size and also attaining normal spherical geometry.

There are now 2 papers that deserve immediate & short term attention:

1. The destructing of erythrocytes in a controlled environment
2. Difference between synth tet. C and human blood.

down the road
3. lineage of harm to health
4. cultured methods
5. mutagenation methods.

Oct 27 2023

The paper he has been written: Cross Domain Bacteria (CDB).
The Destruction of Blood.

Three things on tap:
1. pH of buffer – Carbonate, citrate 8.53 pH
2. pH by alpha lipoic acid 4.67
3. pH by NAC 2.08

Use distilled water.
How would these behave under titration.

Alka Seltzer already ~~titrated~~. Initial pH was 5.9.
It is steadily climbing upon stirring.

There are four (and actually more) ways of making a buffer:

1. Mix a solution of weak acid w/ a solution of the salt.
2. Dissolve solid salt into a solution of weak acid
3. Mix a solution of weak acid with a solution of strong base (such as KOH). The base and some of the acid react & produce the salt.
4. Dissolve solid base into a solution of weak acid. The base and some of the acid react & produce the salt.

The Best so far:

Another source gives another four ways

1. Slow & Stupid Method.
   1. Set up weak acid to occupy ~ 60% of the final solution volume.
   2. Add strong base to reach desired pH (Carefully!)

or in reverse

1. Start w/ the conjugate base and add strong acid to desired pH.

When pH is correct from either method, add water to just under the final volume. Now adjust final pH, then add water to the final volume.

Advantage: Easy to understand. Slow to do. pH control is difficult, but can be.

2. Mentally Taxing method.
   Primarily Computational. Requires the buffer pK and solving two equations. Looks Like the Analytical method.

3. The Two Solution Method: Mix solution of the acid & the Conjugate base w/ same buffer Concentration as final solut. conc.

4. The Completely Mindless Method.
   Use a table of amounts for specific pH or buy prepackaged like I show Below for 2L of phosphate buffer.

studying the behavior of bicarbonate - citrate solution (alkaseltzer) we already kyle agency. the pH has constantly slowly increased over an hour from 5.9 to 8.6.

a buffer also has a pK. you could titrate a buffer, an amino acid, a strong acid, a weak acid, an acid mixture, or a mixture of all the above, any or all.

so how would you know what you have? it seem to me that the composition of the TSP could be extraordinarily complicated, and you could be seeking out

"what it is made of" for months on end.

and then you would still not know how it all put together. this could easily be an extra ordinarily difficult proposition w/ possibly nasty to show for all that staff in the end.

Herpes assessments of primary attributes such a

1. Buffering agent. How do you interfere w/ protein formation? Answer - Enzyme of logical choice and it has shown itself to be the case.

2. Acidic nature: How do you counteract this? With the use of a buffer at the right location. of pancreas. the likelihood is too late in the stem

The study a strong study & be very effective w/ immediate proposed solution.

The direction method as practiced before 9a year on end, can be quite frustrating unless one has the means to put pieces fall the parts together. Pretty much impossible.

As logical action of countermeasure based upon larger picture attributes seems to be what is most effective here - i.e. the big picture vs ever more directed minute detail.

And so the next question is:

1. Is there another strategy - eg methylation? How would you test that?

2. How would you isolate the enzyme of effect?

3. Apply buffer to cultures? Can you demonstrate influence

4. How to spread down polymer in the body?

5. Polymer - alcohol reaction?

These are all big picture items and strategies that can be deployed w/ minimal resources. The is the way.

And so you must focus on what you need to know under the demands of time and circumstance.

The next paper that helps is "blood differences".

Oct 28 2023

Great questions from yesterday:

1. Test methylation ideas
2. Isolate enzyme(s) of my mycanase
3. Influence & Role of Phosphorus in culture
4. Degrade polymer in the body?
   Determine nature of polymers
5. Polymer - alcohol reaction
6. Need — magnets

Now we see that it is weak w/unknowns or mixtures to use titration as an effective identification technique. Essentially everything in solution has a Ka, no matter how complicated the solution is. It tells you very little unless you know your solution to be pure. pKa is nothing more than a log of Ka. You overestimated the value of being able to determine Ka as nothing you have is "pure" or even close to it. Relatively pure formic acid was a dream case, not to be found elsewhere very easily.

—

Now in addition to the wonderful questions above, and the outline of discussions ahead of

1. Blood differences
2. Culture methods - panel
3. Mitigation strategies - panel, market protection

—

Then there brings up the question of chromatography...

What you see in that the TSP is for too complicated to attempt "identification". Chromatography is suited to try and break them down a bit. It make a lot of sense.

You could start w/ the dd Cant just as the already incorporate a major separation. Refractometer useful for initial separation, as well as pH. UV also. TDS also. Useful. Let's try this. Then NIR & ACV.

But remember the very worthy questions.

Now chromatography has different styles
1. Acid - base
2. Ionic
3. pH

What you do not want to do is play up the columns so separating is important. But that you also have the questioning helps track what you are missing on.

I see in one spreadsheet that I used in chromatography HPLC (high pressure liquid chromatography) ... so that it will be LPLC (Low pressure liquid chromatography) and it will alternate to LPC - Low Pressure Chromatography or maybe even further to just LC - liquid Chromatography - make sense.

that I converted all readings to probability values.

I see that I have a full spreadsheet template somewhere - 5 sheets and very sophisticated.
I can use a simplified version to get started.

The spread sheet also shows all my solvents used. The list is quite extensive. Solvents do have a cost and are not easily accessible here. Also impure solvents such as my "HCl" cause problems. Example of solvents used were:
Water, alcohol, acetone, hexane, salt water, bleach

Test the solvent first always before placing it into the column. Controls on the solvent are important. You can run the solvent through the column first and then add the sample.

10 ml is sufficient to
1. Collect PPT
2. TDS
3. pH

} These are quick simple indicators of changes

I am developing LC methods again. Much faster to get a system optimized. DPM by mercaptopo ester is a good quick monitor. Collect standard volume of 1 cm depth (≅ 10 ml). Continuous run on all samples

The is a good system developing. I have a continuous sampling of 120 ml @ 10 ml intervals.

The results of just the first run are interesting.

You can make all pH measurements in one series, then PPT, then TDS etc. The measurement process can

It looks like the LC items has arrived. You may be able to isolate some components here overtime.

I have close to 20 measurements. It looks like:
1. some change took place in Vial 11.
2. Also Vial 11 seems to be different than 8.
3. Also in Vial 11 the pH seems to be change the most rapidly

All Vials except the first are colorless so our only way of detection right now will be UV. Possible ACV or NIR later on.
There is only very H_2O as the solvent. Let's take look @ UV.
Now since #3 is colored, it may be different as well. So our candidates of viability are
#B4 Some Color
#8 Colorless.
#11
These all do have difference to a degree, therefore we do have a separation take place. #8 & #11 are generally similar but they are quite different from one another.

What we see then is that #4 is the first & immediate elution and it has some color to it. It has UV absorbance @ 272 nm and fully qualifies in the protein category and also matches our tyrosine from 274 nm. The fact that it elutes so readily w/ the OH groups also makes sense here.

Therefore we have very good cause to think that we have
isolated a protein w/ the amino acid tyrosine
within it. The has a pH of 7.50, and
immediate drop from the well water of a pH of ~8.5.
So these are actually very valuable to know.

The compound also separate from both the carbonate
precipitate and since it is separate from #8 also
we know that we already have 3 separations in place
which show the very preliminary work.

Now for the item being we can dismiss No 11 since it
was then not soluble in #8. We are therefore now
reduced to

1. The precipitate by Carbonate
2. The first eluate (#4 Vial) which has a strong
   Case for tyrosine
3. #8 Vial, which is different and does show
   the primary absorbance @ 315nm and also
   notice it shows a valley, a lack of absorbance
   in the 270 region. The saga therefore that it is
   not a protein, still soluble in H_2O but not
   as soluble as tyrosine or the tyrosine based
   protein of Vial #4. So what absorbance takes
   place around 315nm?

Vial #8 happen to be in a flat portion of the PAT
and the TDS profile, so it says it was
a stable solution @ this point and was not
under dilution w/ water. So it is therefore a
different separate layer w/ its own characteristics

315nm Synthesized metal oxide nanoparticles $MnFe_2O_4$

Anothe source says Carbohydrates @ 315nm in H_2SO_4.

"Manganese - Ferrite nanoparticles are a current hot topic in materials science due to their high and tunable nanomagnetism, surface reactivity & chemical stability, which make them promising candidates for biomedical applications." Research gate, note Hernandez

In Vial #11 we actually have a very similar UV spectrum match. to a spectrum provided by Zahid Hussain on Research Gate.

There is a good case for ACV.

Now let's look @ NIR 700 - 1100 nm.
Vial #4 has a slight peak (solutions are very weak from LC) has a slight peak @ 1033

Vial #4	1033	Polyfunctional alkyl alcohols (1029) "Ethers and Esters also Containing alcohols) (Recall proposed polymer reaction w/ alcohol)	Nothing else Close enough.
Vial #8	No signal (organic)		
Vial #11	963	Alkyl Alcohols (962)	Nothing else Close.

Although all 3 vials are very dilute and unlab solutions as a result of LC, nevertheless two Vials 4 & 11 both give a signal of an alkyl alcohol. The three also exhibit very strong signal.

Together we have signal candidates for:

1. Protein — USE MID IR Library also
2. Tyrosine as an amino acid
3. $MnFe_2O_4$ metallic nanoparticle & high & tunable magnetism
4. Alkyl alcohols (Polymerization)? MIDR? Esters
5. Acidic

all in just the first trial run w/ LC & H_2O solvent.

There w/out NIR or ACV.

It appears to me that your 10 ml vials would be adequate for:

1. all LC measurements, pH, PPT, TDS
2. UV spectrum
3. NIR (VIS) spectrum (700-1100 nm)
4. NIR full spectrum 900-1700 nm
5. Electrochemistry

Do not forget our water ion testing results.

***	Calcium	**	Hardness
*	Cyanuric Acid	*	Nitrate
*	Free Chlorine	*	Nitrite
*	Lead	*	Iron

The general picture here the same as that of a, with the Carbonate decant:

1. ~~Acid soluble protein~~ Water soluble protein w/ tyrosine (Vial 4)
2. An alkyl alcohol (Vials 8. & 11), esp (Vial 11) ↑ (but also an alcohol) ↑

two separate Components.

—

Your NIK & 40 TSP Carbonate decant looks good.
Repeat & increase concentration by 5X.

Oct 29 2023

Collecting a constant volume aliquot during elution titration (eg 10 ml) is much easier and more efficient than using time + drop rate counts.

A very smooth paced work flow.

Much better to treat the sample when the column during the prep of the run. Then you will have a neutral aliquot @ the beginning.

—

Here is interpretation:
3, 4, 5, 6 Contain Common sample.

1B short term pH increase PPT = Ø.

11-12 TDS plateau not short term. PPT = Ø

The means nuclear signal beyond 3, 4, 5, 6

The implies this material is rather pure.
One take these for a
An acidic protein w/ tyrosine.
(UV) (274)

Now the pK could be helpful in determining the net charge.
Acidic amino acids are aspartic acid & glutamic acid.
Acidic amino acids like water but so are basic amino acids.

Therefore: There should be four peaks

1. Combine 3, 4, 5, 6
2. UV-Test - Spectrum
3. Use existing NIR plot to interpret
4. Make additional NIR plot
5. Electrochemistry
6. Titration - determine pK.

UV Spectrum.

We have a single well defined peak w/ decent absorbance magnitude at 272 nm.
Either tyr or tyrosine.

w/ VIS-NIR

I have two possible signals:

~1080 nm Alcohols as R-C-O-H (1065) Nothing else around

963 nm OH Alkyl Alcohol (962 nm) Nothing else around

So 1. Acidic (pK?)
2. Protein
3. Tyrosine
4. Alcohol

Do not dismiss yesterday LC run that showed absorbance @ 315 nm. Possible $MnFe_2O_4$ nanoparticle - magnetically tunable.

Titration now be hydrolysis interest as materials appears to be water given by L.C.

The pH of the 3, 4, 5, 6 Vial LC combo TSP Carbonate decant is 7.30.
The pH presents challenges for titration
We have limited material to work with

TDS is 6480 ppm it is reasonably concentrated.
The low boiling material must titration require some additional work to purify.

You also want to work w/ electrochemistry, titration & NIR on the sample

Now notice this sample is NOT acidic in any fashion in fact it is close to biological pH.
The procedure is

1. Carbonate precipitation
2. LC purification.

The same the acid combination may well be Carry from the precipitate and will have been some signs of this already.

So open pH on the alkaline side.

Mass of sample = 28.5 gms.

Let's split it up. Start w/ ~ 5gms and add 25 ml H_2O.

5.0 gms TSP from LL TDS = 1520
+ 25.71 ml H_2O pH = 7.63
30.71 gms total

Use 50:1 NaOH Titrant.
50:1 was perfect titrant.

We have an equilibrium point @ 6.55 ml (pH=
½ Eq = 3.275 ml pH = 10.2 exactly what
vol pH == it got cloudy.
3.10 10.2

We have seen the before.
3.70 10.18

$K_a = 10^{-10.2} = 6.3E{-}11$

?? Who or what has a K_a of ~ 6.3 E-11 and a pK_a of 10.2?

* It also should have a pK_{a_2} like this high.

Carbon. a pK_{a_2} 5.6E-11

The titration look to be valid to me.

Oct 29 2023 (cont) NIR TSP Carbonate Decant LC

I now have a decent LC-TSP NIR Carbonate Decant Plot. The sample should be fairly pure. Four main peaks (1) Weak (3) Strong

Valley — Not Absorbed
1149 CH Aromatic (1143)(2)(1142)

Peak — 1202 OH from Water (1200) Does not seem realistic, should be thoroughly dry
Methyl (1195) This must be considered more probable.

Valley — NOT Absorbed
1334 NO CRC 2012 Listings

Peak — 1376 (1) Weak Methyl, associated w/ Aromatic CH_3 (1370)

Peak — 1499 (3) Aromatic Amine (1496.5)
Note tyrosine already identified w/ UV
Alcohol or Water (1500)
Note that tyrosine has two OH groups
NH Amide, NH or NH_2 Amide/Protein 1500
OH Alcohol, Alkyl Alcohols 1500
Aromatic Amine 1502.5

Peak — 1581 Alcohol R-C-OH (1580)
Alcohols or Water (1583)

So: Aromatic Amine
Alcohol Groups
Methyl
Protein
} Consistent w/ UV and high Solubility.

The purified Component is one that you want to show enzyme inhibition upon. Tyrosine, protein, soluble, methyl would then be a org a target to seek disruption.

* You pk dell/mail (a yr org just up another in the acid
* side is most likely attributed to the protein itself,
* not some other acid a component. Then where the pk
belongs to.

Electrochemistry will tell you more about the inorganic aspects of the protein.

Chaotg on the end will be the enzyme disruption (up from, H2O Soluble)
Buffers will be the stratg in the acid precipitate protein.

Oct 30 2023

Polarity is the main variable/key in the eluent being used for LC. Common sequence from less polar to more polar but "reverse phase" is also a mainstay. All the essentials of an effective simple LC system are in place.

A rather interesting topic arises w/ the recent alkalize titration that has taken place along w/ the determination of a pKa @ pH of 10.2

There is the additional slight amount of precipitation that forms. This could easily be a separate protein from that previously precipitated.

There is the prospect that the TSP may have yet several constituents within it that can likely be separated.

TSP Native Solution
1. Initial precipitation, via Carbonate or base — "Decant" + solution 1
2. Remaining solution, precipitate again — $_2$ + solution 2

There are 2 solids and two solutions as a minimum. LC may be able to operate with purifying on the two solutions?

Let's run a UV spectrum on "Solution 2" acquired by centrifugation of alkaline pKa runs and compare it to "decant" of the initial TSP precip process w/ Carbonate or Lime.

—

Now recall that this "Solution 2" is extremely weak & began with. The decant was diluted by a factor of 5 & 1. Then we titrated the solution and diluted again by a factor of 2. So detecting any thing will be difficult. In addition the amount of precipitate gained in the quest to identify a pKa 2 is extremely small.

Nonetheless, the UV spectrum of Solution 2 shows the following. There is a small rise in absorbance @ ~ 277 nm. The once again tells us that a very small amount of protein exists in the solution. Note here is most the solution ended up @ a pH of ~ 12 vs 10.2 where maximum insolubility is expected at the pI 10.2 point. So the curve is a return of a portion of Protein 2 (ie precipitate 2) into solution.

Protein 2 Solution

But also importantly, the peak @ 272 nm does not show up, this indicates that the tyrosine aspect has been removed @ the time, or at the stage of separation.

—

Next we want to think about the very small amount of precipitate 2 that we have collected by centrifugation. We need to get it back into solution for UV work (not so for NMR work later) so the solvent it should be hydrolyzed (ie dissolved) by acidifying. We'll add 100 uL H_2SO_4 to the precipitate that has been centrifuged.

Our UV blank H_2O will also need 100 ml of H_2SO_4 as a reference.

OK, then has worked very well. I have plenty of precipitate for UV analysis via centrifugation of the pkg tube, and it easily hydrolyzed with 50 ml of H_2SO_4. It is beg reference to H_2O w/ 50 ml of H_2SO_4.

Power went off. But there is a discernible peak @ 280 nm.

Evidently we have a second protein here.

Precipitate Protein 4 ~~Protein 3~~ Solution 2

Repeat the UV run.
OK, a definite discernible peak @ 280 nm.
Two separate precipitated proteins determined to exist within TSP, separable by Mr.

Primary solution after precipitation also undergoing investigation, and it says a third water soluble protein as given NIR analysis:

Water Soluble Protein

Protein 1

1. Aromatic Amine
2. Alcohol groups - alkyl alcohols
3. Methyl
4. Protein
5. Tyrosine

Decant 01 — Solution 1 – Protein 01
Precipitate 01 — Solution 1 – Protein 02

Titration pk2 — Solution 2 Protein 3
Titration pk2 – Precipitate 02 — Solution 2 Protein 04

There are therefore 4 potential proteins subject to isolation in the native TSP solution alone.

Settled Protein — Protein 5
Filament Protein — Protein 6

The bunge & G the number of identified candidate proteins.

The next logical step is to see if a pK can be identified on the acidic side using TSP Carbonate decant. Same process. Set 5 ml decant + 25 ml H_2O. Titrate w/ H_2SO_4 50:1

We Can make ~ 200 ml of solution 200/50 = 4 ml H_2SO_4
to use 4 ml H_2SO_4. Add 230 ml H_2O → Total Volume 204 ml
Mass of vessel = 34.83 gms + 4 gms H_2SO_4 = 38.83 gms

Mass of vessel 186 gms + 200 ml = 386 gms Superceded in part

Ok, acid was too strong then by a factor of 20/4 = 5 run
We need to dilute to a ratio of 250 to 1 vs 50 to 1.

Nevertheless, we may have an pk estimate. Eg pt = 0.10 ml
½ ml = 0.35 ml → leads to pKa estimate of 5.29
$K_a = 10^{-5.29} = 5.13E-6$
But let's dilute.

I wanted to add 205 ml made it to 190.

Had 41 ml of 50 to 1 H_2SO_4
added 190 ml of H_2O to above.
Total volume = 231 ml vs 250 desired
$\frac{231}{50 \text{ to } 1}$ = 4.62 ratio in our found ratio's

4.62 (50) = 231 to 1 vs 250 to 1
It can be ~ a 230 to 1 will work OK!

OK, there is a suitable titration.
We have a minimum slope @ 1.67 ml (pH = 4.89)
1/2 (1.67 ml) = @ 0.835 ml pH = 6.25

Therefore we have a pKa_1 @ 6.25
or Ka_1 = 5.62E-7

and we have a pKa_2 @ 10.2
or Ka_2 ~~5.0E~~ 6.3E-11

This says to me that we have two different proteins in the precipitated de Carbon Carbonate TSP.

1st precipitable — One with a pH of 6.25 = pk and Ka = 5.62E-7

and

2nd precipitable — One with a pk of 10.2 and Ka = 6.3E-11

You will need to think about the a lot to interpret and to retrace the many steps that have been involved.

There value make sense in that you have not to different precipitations that take place right @ the pH value.

It is also of interest that we see another monotype that is probably @ 4.25 ml 1/2 = 2.125 ml
pH = 4.09 Ka = 8.13E-5

The means ya allow for another entity, protein, acid, metal, etc @ a Ka of 8.13E-5.

The suggests the TSP has three diffent pka's in solution.

Interpret pka as a function of proton charge.
Verify the work. Work.

Oct 31 2023

"pA" determination w.r.t. proteins is actually the pI.
Isoelectric point.
Each pI being determined identifies a unique protein, especially as is evidenced by a precipitation event.

We have 2 pI's identified and now a possible third.
The work is to be replated. It is of interest that one pI is basic and the other acidic, The actually concern w/ the bipolar distribution of charge on proteins so I shall use methods of adding base on one side and acid on the other sound.

Now we have the question, Can we work through this process w/ out including chromatography? The chromatography essential @ the point as we do not have confirmation of any real separation taking place thus under H_2O eluent. I will try this along with filtering.

We have reliable varied production within a ~~H_2SO_4~~ NaOH precipitated TSP culture that has been sitting idle since Oct 20 and the pH was set to 7.4 @ that time. The culture is not incubated and temperature is let are gentle cool - down to 40° C night.
We have
COB, COB clusters, protein conglomerates, Crystals and fairly generous synth cell production. Synth cells are small, probably on order of 4 microns diam. Incubation may be a factor here. Check on pH.

Ok a very big surprise here. The pH has increased from 7.4 at day precipitation to 8.6. I do not think this has been seen before.

It is a very productive and stable culture. Very representative as well.

Now observing TSP 51, 52, 53 series
TSP 52 & 53 and a pH monitor for both have been incubated continuously since approx Oct 20 as well.
Now the pH of the monitor is ~ 5.4

What a big difference we see here! It is a good question as to why. Now let's look @ 52 & 53. 52 had some human blood added, 53 did not.

Incubated cultures do have a detectable odor.

TSP 52 has fewer but still many synth cells
They are multi layer - full size. The more mature and larger and uniform they are, the more likely they seem to contain a solitary central COB. Vacuolation is size makes the case for synth cells.
No Crystal Structure. High Protein Level.
Now let's look @ TSP 53.

Synth cell production w/ common central COB same as in TSP 52. Appear to be fewer in number than TSP 52. Slowly slide gently produces least vacuolation.

The observations of these culture states today says

1. Incubation appear to be important and necessary for synth cell production

2. Synth cells seem to be able to survive in pH as low as 5.1

3. Central COB is common w/in mature synth cells.

4. Incubated synth cells are full size: 6-8 um most common.

5. No crystal structure, high protein content likely. No filaments observed in incubated cultures.

6. Gentle side flow of cover slip improves clarity of viewing considerably.

7. Incubated cultures appear reasonably uniform in appearance; no obvious surface light development when crystal layer develop.

Return to incubation. Now let's incubate in TSP Series 60 also (large volume, pH up to 8.6) Varied culture, small but numerous synth cells on surface layer:

Ok, a rather amazing observation and event has taken place.
Designate it as TSP-70.

Massive Synth Cell - Polymer surface layer production.
It is quite astounding. This is a large volume culture that is not incubated. Very pure white polymer synth cell production on surface layer continuous over circle of ~7" diameter. It is a white polymer layer saturated w/ synth cells and synth cell massive production centers.

The well is very suitable for hemoglobin testing.

NIR Card has been made, pH is 4.6
The material dries fairly quickly - relatively so.

The culture must be preserved to the degree possible

I now have a clean NIR comparison plot between human blood (as it exists on the Blood Destruction paper - relatively normal appearance now) and the synth blood. There are starkly differences.

NIR Human Blood 98.2

921 Very high absorbance

Now the big picture here is that CH_2 is dominant in the region, w/ a possible ROH Just ROH is centered more on 960 nm by CRC vs ASD shown 920

CRC shows Methyl and Methylene dominant here.

In all cases blood the Component seems relatively abundant

IR plots of blood sampled in 2015 -2017 show strong absorbance @ 2920 - 2940.
This is RCH_2CH_3 alkane

We now have recovered Hg Blood IR Plot Nov 2015
Prominent Peaks @ 3286 Carboxylic Acids
2919 Alkanes RCH_2CH_3 Carboxylic Acids RCOOH
2857 Alkanes, Carboxylic Acids
1646 Amides, Alkenes
1536 amides
~~1296 Amines, Carboxylic Acids, Esters~~

Wavenumbers
MID 2015
IR
VOID!

Amines C N bonds
Amides have a Carbonyl group attached C=O

NIR 983 OH from water (979) possible - you would need to test this

NIR 1415 Methylene. CH_2 (1415)

NIR Synthetic Blood NIR

The synth blood shows very low relative absorbance in the alkane group.

—

Spectragraph is about the most non-intuitive IR viewing program possible, but I finally got it to work. To get peaks, you need to click on the wrench → Peak values. Then you need to

1. Erase anything
2. Reset everything
3. Add Custom labels.

It is screwy as all get out.

NIR 1199 Methyl . CH_2 (1195)
Water (1200)

NIR
M416 Polyamide (1480) No Counterpart in human blood
Aromatic Amine (1412)
Secondary Amine (1481)

NIR
~1519 1580 Alcohol (could explain why it dried so quickly) No Counterpart in human blood
1583 Alcohol
1510 Polyamide

Human Blood

NIR	1523	Amide/Protein (1520)	
MIDIR	1646	amides	
	1536		
NIR	1627	Vinyl, Vinylidene (1630)	ArCH indicated
		Vinyl (1621)	from general NIR chart
Nano meters		Vinylidene 1631	by Galaxy Scientific

Now note that health impacts were very much in place in 2015 so it become very difficult to sort out "reference blood".

The Mid IR data indicate that a Vinyl attribute may already be in place in blood of 2015.

Polyamide & alcohol are two peoples in the synth blood over human blood.

Now, the material under examination by NIR is not soluble, so an aromatic amine does not make a lot of sense.

It be the appearance of a polymer. Polyamide is a logical choice and alcohol from the drying nature.

There are alcohol soluble polyamides Polyvinyl alcohol is a polyamide

Nov 02 2023

Some good things in place here.

First, notice that the TSP-10 culture has always been covered w/ a somewhat tight fitting lid. Whether or not this is a factor in the unusual development taking place is unknown, but it should be mentioned. Then introduces the question of Oxygen availability in the culture development and the effect of that variation.

The appearance of TSP-10 (not to be disturbed for now as it is so unusual) has an uncanny similarity to that reported in the paper "Frequently Produced Disease?" which in itself was a most unusual occurrence. Filaments are not obvious @ the microscope level (synthetic blood in) however @ the macro visual level there is clearly a filament nature that has appeared. At this point, components would appear to be:

1. Mace filament structure
2. Massive synthetic blood production (micro level)
3. A polymer surface layer
4. An alcohol is in question because of NIR analysis

It is a most unusual and remarkable culture.
It has the making of becoming the equivalent of a 1" drain surface layer "blood clot" if synthetic...

I have some other things going on here.

1. I now have hydrochloric acid on site. The new test is a bit more work and time than I ever expected. We are all very protected by "regulatory interests" these days, and it takes some real effort and ~~cost~~ Cash to acquire basic chemicals for lab operations. It is a constant struggle but I do have some basics in place now.

—

I have hemoglobin measuring capability now, at least directly from human blood. It is electrochemically measured via a meter & test strip, so I am not sure if it will apply to culture investigations or not.

—

Another big achievement: Even though I no longer have access to my prized MIO infrared instrument in storage in VT, (maybe someday?) I have been able to reestablish access to my digital spectra library acquired over years of on-hand work w/ the last instrument. I likely have 1500 or more scans along w/ a commercial library of ~5000 scans. This is therefore another great asset.

Of course, it would be most beneficial to get the actual instrument in operation again, but that still remains an unknown at this point. The instrument is no longer sold and therefore also unknown if it can ever be repaired.

My gate date back to ~ 2015-2017.

In now the strategy is to combine current NIR work w/ the historical MID IR work to look for what level of correlation exists.

It does exist as a valuable library of additional interpretation.

I have just completed a hemoglobin test w/ the water acquired.

My result is 13.4 g/dL gms/deciLiter (Hematocrit (40.2%) (38.3-48.6 adult men)

The normal range for men of the middle age is 12.4 not Senior Citizens
to 14.9 ($\bar{x}$ = 13.7 gms/dL

I am, therefore, extremely close to the average value.
This is, therefore, a very good result.
For adult males, (< middle age) is 14-18 Gm/dL.

Another source American Family Physician, propose the following lower limits.

		Age		
Men	White	20-59	13.7	The source places me
		≥60	13.2	@ the lower limit of
	Black	20-59	12.9	an acceptable range
		>60	12.7	
Women	White	≥20	12.2	
	Black	≥20	11.5	

This is, therefore, very meaningful.

General population has anemia @ 17% level
Community Dwelling older adults in 7-11%
Nancy Lowe 47 yrs. (!)
Hopd Hospitalized patients 40%

Mild anemia is considered @ 11gm/dL
However, even mild anemia is associated w/
increased mortality.

The hemoglobin meter does give a result
* positive for hemoglobin but below measurement
specification of 7 gm/dL. — w/ the culture TSP TO

Positive H_2O_2 - Catalase / Catalase test.

My hematocrit reading is 40.2% also a bit
low.

There is a YouTube video that shows the meter
reading low relative to a certified lab. Keep in mind that Cathelicidin can become a
mine.

I will repeat the test.

They may have just multiplied the hemoglobin
value by a factor of three, as parent a
standard rule of thumb. The meter might
actually only measure hemoglobin

Hematocrit can, in fact, simply be "defined" as three times Hemoglobin so it fails as an actual measurement. Not a worthwhile system

—

My second reading came out much too low, just like the YT video used @ 11.5. The doc not look reliable

Next time it measures 13.7 & 41.1% (they are just multiplying by 3)

—

Ok, I think that the meter is gradually reliable. I will only count one reading as erroneous.

(Expected range for venous male 12.4 - 14.9

13.4

13.1 noticed many males 7-60

11.5 regarded as erroneous

The unit is measuring temperature on the sheet.

This actually is extraordinary. I have indeed been able to capture a hemoglobin reading from the TSPTO culture on 10.5 gm/dL.

The quite angry. Hemoglobin has been verified again, as it was many years ago. The card, however, is with a digital test meter.

There now in addition to the H_2O_2 positive catalase result.

Let us now see if we have the Kastle-Meyer materials.

Well, I finally now have a second sample calibration solution set for pH control. These very helpful as accurate pH control has now become critical.

Two point calibration led to pH meter of 0.3 on far end of alkaline scale. All is in range now.

Kastle Meyer sequence
1. Water on Q-tip
2. Blood on Q-tip → 3. Ethanol
4. 3. Phenolphthalein
5. 4. H_2O_2

Well, I have a presumptive result here. The fresh, live blood failed the Kastle Meyer Test — how can that be?!

OK, good! I have the Kastle Meyer test working now. Some of the methods online given are inadequate.

Kastle Meyer reagent must be prepared! It is not just phenolphthalein. The reagent phenol — to be placed in an alkaline solution which will turn the solution a dark red. Then if you use the test you will see the color change from the dark red to a pink color as it oxidizes and is tested as a positive test.

Then put in 9 white pond 03 2023

Nov 03 2023

✓ 1. TSP 50 dated back to 10/19 now seems to show a visible polymer layer - need under scope.

In Progress 2. Am attempting to dissolve nylon in HCl solution (polyamide) (dilute)
Also in H_2SO_4 (dilute)

✓ 3. Kaitlin Megla needs investigation

✓ 4. TSP 70 seems to have devolved last night but overnight seems to have reformed on surface. It is a remarkable synth (blood) cell culture.

The pH monitor for pH has now settled in @ 8.4
and the pH of TSP 50 was measured on Oct 31 at 8.6
There now a yeast and mould culture w/ a replicate active
Contribution as noted on Oct 31.

I will now dispose of TSP 50 #57.

Next I am looking @ TSP 53. The culture is now slowly drying up having been in the incubator since ~Oct 24 w/out replenishment of fluid. The pH of the monitor is ~ 4.8.
The pH measurement is of interest in its own right.

What we see in TSP 53 are ~~Grey~~ Crystals that probably resemble the crystals in the Exotic Crystals paper more closely than any they seen since. That culture was the harbinger to the first synth cell appearance.

The pH range is now varying between 4.5 & 8.5. The culture results are varying accordy to both temperature and pH and they should come as no surprise since ~~at the~~ one of the tenets of Biology.

The Crystals that have appeared in TSP53 come from an inoculated culture since Oct 24. Temperature is therefore presumed to be a likely factor in how and ~~how~~ they formed. Ambient temperatures were much warmer when the "~~Exotic~~ "Exotic Crystal" paper was written, and there also was a the giant mystery ~~Erythrocyte~~ appearance happened - ever cleared red in color. It looked like blood and showed itself to be blood.

Therefore temp and pH monitory is required to sort out the many culture variations that are showing.

Some parties now designate the CDB as "micro bots ~~bots~~". Nothing like adding to the confusion as also with "Kinetically Activated MicroStructures". I guess everyone needs their own name. CDB was used by the greatest delay & hesitation over a lapse of a decade or more, and at my necessity.

We may now ask:

1. Does a robot have DNA? Do Robots grow from blood?
2. Does a robot produce 18+ Protein expressions cultures
3. Does a robot vary its Protein expression per pH and temperature?
4. Does a robot produce synthetic blood?
Possibly, but not as likely, the laws of biology still prevail @ the stems, Even w/ synthetic biology @ the helm...

I will now dispense w/ cultures TSP 52 & 53.
" " " TSP 60.
Nothing new learned here.

I start the new cultures. It will now be
4500 ml 4.5/1.7 = Factor of 2.65
This means:
4 tbsp sugar (2.65) = 10.5, call it 10 tbsp sugar
10 tbsp fertilizer (SPONGE) ~~2 tbs~~ 5 tbsp starch
1.25 tsp pink salt
a drop of blood will do it, but a few help even more.
& extended the culture will take about 3 days to get fully active. Monitor VOCs.

In addition I will make the 1.7 liter culture
4, 4, 1/8, blood 2 tbsp starch

Culture started.

The HCl & H_2SO_4 is not dissolving nylon samples quickly by any means. Need to allow time.

What is most confusing @ the point is that the Kastle Meyer test is failing, even w/ a known live or dried Bloodstain sample.

This is most puzzling @ the point. Why?
1. Improper procedure
2. Faulty reagent(s) or wrong reagent(s).
3. Blood has changed

I certainly do not have any recollection of such a difficulty in the past. The test performed exactly as expected.

Why? * In Contrast, the sample after treatment is NOT turning pink. Very perplexing. H_2O_2 activity by Catalase is observed.

~~This~~ Kastle Meyer test relies on the "peroxidase-like" activity of blood to catalyze the oxidation of phenolphthalein

If the test is negative it is reasonable proof that heme is absent in the sample

Kastle Meyer solution may not just be phenolphthalein.

One source gives ingredients as 2% phenolphthalein in 20% KOH.

I now how the Kastle Meyer test works on live blood - dried. See Corrective notes in margin for ~~Oct~~ Nov 03.

—

Ok, the preparation of the Kastle Meyer reagent is important. Ideally you add heat to phenol — and then look at it w/ zinc. There is a reduction that takes place and it turns the reagent yellow orange.

If you do not have zinc, do not ~~t~~ see that a reliable test occurs also if you add about 3 full eyedroppers of phenol — to about 10 ml of H_2O with 1 drop of strong KOH - NaOH. You need to turn the solution a dark red to be able to detect the change to test pink.

—

Your Kastle Meyer test is not as good as you would like it to be. It may be fine as a definite shift to pink is observed, but you would like your reagent to be colorless or yellow form (ie, reduced)

Actually Phenolphthalein 2% in 20% KOH is also they sold as Kastle Meyer reagent.

—

You can get a suitable Kastle Meyer test result under the following conditions:

1. The KM reagent must be prepared as dark acid as possible if you do not have zinc available.

2. If you add too much base to the reagent, it will actually turn light in color and then eventually colorless.

3. It would seem the pH for that will be in the range of ~9-10 vs 11-12.

4. Now if you run a control of just the phenol— and H_2O alone it will also turn pink, however if you add a second drop (or very, very small amount) of H_2O_2 you will see that the H_2O_2 will eventually bleach the phenol— to colorless.

This will not be the case with a positive test result. It will, just as I said, change from the darker red test by phenol reagent to the bright pink expected. The difference w/ a positive test is that it will remain the bright pink color no matter how much of the phenol or H_2O_2.

The have been tested w/ both the dried blood sample and the TSP 70 culture. The control w/ phenol— & H_2O_2 was also made and by me.

There is now high confidence that the TSP-70 has indeed produced a positive Kastle Meyer test in addition to measured hemoglobin w/ the digital meter.

It would be beneficial to have commercial KH reagent that separates the Cola particle to yellow, but it is not required if these [illegible] conditions are followed.

The longer you wait w/ phenol — + KOH + H_2O_2 the more it will become white and colorless. Not the Case w/ a positive test; it will remain pink. Then ready to sort out.

The topic is now suitable for writing:
Cross Doorman Backers - Synthetic Blood & Hemoglobin Confirmed

Polymer
Alcohol
Protein
Hemoglobin
UV is available as flash drive 272nm

Human Blood & Synth Blood

Nov 03 (cont) 2023 NIR & MID IR JOINT ANALYSIS:

I have now combined two sets of human blood NIR and synthetic culture erythrocyte two sets also. They are similar enough that the process can be justified. Let's make another comparison from scratch

(1) Weak (3) Strong

	Human Blood		Synth Blood
Valley	914(1) High Magnitude Methyl (915)	Peak	914(1) Low Magnitude Methyl (915)
Peak	932(1) High Magnitude Methylene (930)	Peak	934(1) Low Magnitude Methylene (930)
Peak	999(3) Primary Alcohol (996) Aromatic Amine (1003) Secondary Alcohol (1054) Tertiary Alcohol (1006)	Peak	1000(2) Primary Alcohol (996) Aromatic Amine (1003) Secondary Alcohol (1004) Tertiary Alcohol (1006)

This group presents a puzzle. Neither is expected?

Now let's look @ some external reference sources

1. Research Gate: Morabito lists MID IR: CE 2015
   1. Water and OH (3500-3200)
   2. Amide (3284)
   3. LIPIDS (2958)
   4. LIPIDS (2872)
   5. Amide I (1700-1600) C=O
   6. Amide II (1560-1500)
   7. LIPIDS & PROTEINS (1396)
   8. AMIDE III 1239 (C-N)
   9. Glucose 1082
   10. Amide IV (C-H) 698

1310 matches primary reference no. 2.

3286 Carboxylic Acid?
2920 Alkanes, Carb Acids
2851 Alkanes, Carb. Acids
1646 Amides
1536 Amides
1298 ~~Carb. Acids~~ Ester Amines

also ~1310 OR ~1161

VOID

THIS MYSTERY HAS BEEN SOLVED.

1296 cm^{-1} DOES NOT EXIST ON BLOOD MID IR NOV 2015

Notice neither source is stating aromatic amine - Possible OH reference. but alcohol not stated. Would be required C be a soluble aromatic amine. Tyrosine is strongest candidate as being identified in UV direct @ 272nm.

2nd Source Consulted Applied Spectroscopy Dec 17 2020

Again no 1296 peak stated. 1286, 1308 assigned to proteins

again No aromatic amine or alcohol stated, even a/a in fingerprint section

Detailed peak listing & assignment.

3rd Source: NIH.gov Fadielmoula Jan 2022 — THIS NOW AGREES WITH DATA

1645] listed 3286 ✓ 2920, 2857 ✓ 1646 ✓ 1536 ✓

1551]

CI 1296 Repeats as a question, not listed

4th Source NIH Shanshan Dec 2022 Digestive Cancers

Discusses nearest 1309 cm^{-1} to 1296 as related to CH of nucleic acid.

1296 cm^{-1} remains an anomaly after 4 sources

No source mentions alcohol as a significant component or aromatic amine

It could be both are present since absorbance is so high and broad

Note: DEC 06 2023

1296 is incorrect. do exist in human blood in Aug 2015

1310 & 1160?

Continuing:

		Synth Blood
Non existent	Peak	1196(2) Methyl ((1194) (1195) Suggests a displacement of methyl
(valley) Non existent (valley here)	Peak	1364(1) Methyl (1360) Suggests a methyl displacement

Peak 1413(2) Methylene (1410) *

Alcohol (1410)

Methylene (1411)

Alcohol (1415)

	Human Blood	Synth Blood
Peak	1474(3) Aromatic Amine (1472) Amide/Protein (1471) Methanol (1470)	
Peak	1523(2) Amide (1520) / Protein * Secondary Amine (1520)	
Peak		1582(3) Alcohol (1580) Alcohol 1583
		No real competition
Peak	1628(2) Vinyl & Vinylidene (1630, 1631)	
Peak	1691(2) Alcohol, Diol (1680) CH Aromatic (1689) Protein (1690) Methyl (1693, 1694)	

Therefore:

1. A water soluble aromatic amine
2. An alcohol

become prime candidates in the synth blood

In addition within the blood

1. Vinyl & Vinylidene are investigative candidates
2. Unidentified 1296 cm^{-1} peak raises questions

Nov 04 2023

There are now several strong leads to pursue from the comparison of human blood vs synth blood:

1. Aromatic amine? 8. Alcohol reaction w/ TSP explored.
2. Alcohol?
3. Tyrosine
4. Polymer
5. Vinyl — NOT Actually solved properly on the second round!
6. ~~1296 peak CH~~ — VOID — ~~MYSTERY~~ SOLVED (see note below)
7. Comparison of historic blood spectra

—

HCl & H_2SO_4 (both fairly weak) soln with Nylon — not dissolving.

Qualitative testing? Titration repeat w/ HCl.
Chromatography?

I would like to clarify the above briefly before proceeding w/ synth blood research paper.

—

Next Sunday. Strong HCl & strong H_2SO_4 both dissolved Nylon quite quickly and well. Weak solutions do not. H_2SO_4 is superior. The answers an important question for UV preparation of any polyamide. This is very helpful.

—

The 1296 cm^{-1} problem has been solved. It simply does not exist on the 2017 CE blood plot. I am not sure where the mistake originated from other than potential confusion w/ 1296 nm on NIR?
There is good news as MID IR info recorded is now congruent w/ NIR IR.

Actually solved more completely now. See notes for Dec 06 2023

Testing for alcohol:

1. Test strips
2. Refractometry
3. UV not usable - Isopropyl has peak @ 207
4. VIS-NIR?
   There is some NIR-VIS activity 960-1010nm
   Let's try this w/ isopropyl & ethanol.

Alcohol floods or wakes also.

I am doing some calibration work w/ UV on the detection of alcohol. I can most definitely distinguish the presence of an alkyl alcohol, namely isopropyl alcohol.

The method requires that you use the same cuvette for both the control and the sample. The elemental mismatch in cuvette variation that effect the light absorbance magnitude that is to be measured. Nevertheless, the alkyl signal @ ~ ~1050 nm can be easily identified.

There is now a modified method that can be used for very sensitive or low concentration UV work as it eliminates the most important error of cuvette variation (~.003 Au)

Detection level now is @ ~ 3-5 drops of 90% isopropyl ~~alcohol~~ alcohol in ~3 ml of H_2O.

Isopropanol peak was @ ~1049. (alkyl alcohol)
Ethanol peak is @ ~1032. (Polyfunctional alkyl alcohol)

Notice that the alcohol is denatured so it is likely related to the shift in primary absorbance peak. But they are both alcohols.

Ethanol peak almost 10 times stronger than isopropanol.

Now let's part detection levels.

200 ul shifted the propanol peak to 1024 (vs 1029) (now even for isopropanol). But the peak remains clearly identifiable.

100 ul become a very borderline situation to just try to keep the concentration level higher if possible.

Now a run of TSP TO from under the surface. Definitely NO absorbance in the 900-1100 range on the run. This does not mean that there is no alcohol, but it does indicate that there is not an alkyl alcohol under the surface.

Now let's look @ the upper layer. Is it soluble? If so, in what?

Ok, the top layer is not exactly soluble in H_2O.

Very interesting.

Not soluble in 50ml HCl in ~1.5ml H_2O

Not soluble in 50ml NaOH-KOH in ~1.5ml H_2O

Possibly slightly soluble in ethanol 50ml in ~1.5ml H_2O

Next in acetone:
~ 4-6 drops in ~1.5 ml H_2O.
(Seems to have made an unusually strong vacuum after shaking).
Seems partly soluble similar to ethanol

The material, presumed to be a polymer, is becoming interesting. It is not readily soluble in common reagents, albeit sparingly.

Polyvinyl alcohol is soluble in water.
This is definitely not.

It is the least soluble in NaOH-KOH

* Completely insoluble in gasolene. Therefore partially polar

It appears to be soluble in strong H_2SO_4 (~50% H_2O) and also is generating a lot of heat in the process.

This is going to be useful as it separates from alcohol

Yes, definitely soluble in strong H_2SO_4.

It actually appears to be soluble in acetone eventually also, and this is very important, as it was also relatively weak.

This is likely also going to be very useful in LC separation. $H_2SO_4 + H_2O$ alone generates a lot of heat by itself, so hard to separate out anything there.

OK, this study is becoming very extensive. We have in place the means for light control and UV low detection protocols.

The investigation of TSP-10 surgical polymer dissolved in H_2SO_4. The control of both H_2O & $H_2O + H_2SO_4$ are in agreement. The current range is 900–1100 nm.

What we see in absorbance is the ~900 region. Let's extend range from 700 to 1100.

The reference will be H_2O + 300 µl 50% H_2SO_4
Sample will be above w/ TSP-10 surgical staple.
Range will be 700 – 1100 nm.

It is rather clear where things are headed. There will be a strong explanatory comparison between the clot which dissolves in H_2SO_4 and the TSP-10 polymer, which also dissolves in H_2SO_4.

Watch this statement, I think clot dissolves in KOH–NaOH

12/06/23

Jan 27, 2024: This work must have been done w/ Nirvascan.

I now have a very clearly defined VIS-NIR spectrum of the TSP 70 surface polymer with well defined peaks and well defined valleys & shoulders.

TSP 70 Surface Polymer:

(Weak)(3)Strong

Peak(1) 1097nm(3) Alcohol as RCOH (1065) No real Competition
xx ArCH (~1090) ASD Chart (Perfect Match, No Competition)
CRC does not have this until 1142nm (Important difference)

Valley 1040–1070 Methylene, Alkyl Alcohols (CRC) (UV run confirms
No Activity per ASD Chart (absence of alkyl alcohol)
(recall insolubility in gasoline)

Very weak Peak(1) 958 Alkyl alcohol w/no Hydrogen bonding (CRC) (962)
No activity except H_2O per ASD Chart

Valley 914 Methyl (915) CRC (recall insolubility in gasoline)
Methyl - ASD Chart

Sharp peak(1) 875 CH Aromatic (876) CRC No Competition
xx ArCH – ASD Chart, Possible Methyl - ASD Chart

Valley 855 No activity - CRC
No real activity - ASD Chart

Potential Peak Area 706 CH Aromatic (714) CRC
Flatter xxx ArCH, Methyl ASD Chart.

We therefore have a strong determination of ArCH as well as a likely alkyl alcohol w/ no hydrogen bonding. → Look this up!

Will need to learn what the latter structure is.

It make sense that it does contain some type of alcohol as it dries quickly. It also makes sense that it is ArCH since:

1. polymer nature
2. Polarity behavior somewhat unusual
3. Acid base (strong) ultimately important for solubility
4. Hydrocarbon development seems moderate due to solubility behavior.

"As the length of the alcohol increases, the solubility decreases.

The physical properties of higher molecular weight alcohols are very similar to those of the corresponding hydrocarbons

"Dendrimer" chemistry may well be at play here.

Solubility ionic and behavior are central to what is being discovered. Initially water soluble, then insoluble in between.

The longer the carbon chain in an alcohol, the lower the solubility in polar solvents

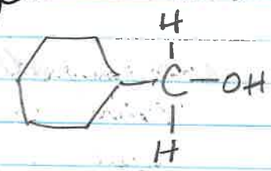
The alcohol 1-decanol is essentially insoluble in water.

$CH_3CH_2CH_2CH_2CH_2CH_2CH_2CH_2CH_2CH_2OH$

Alcohols of higher molecular weights tend to be less water soluble.

The smaller alcohols
methanol, ethanol & propanol dissolve easily in water.

An alkyl group is formed by removing one hydrogen from the alkane chain.



Nov 05 2023

One of the problems shaping up is the attempt to develop a model of the major constituents of the TSP. It will be tricky to keep track of the many contributing processes & sources of information coupled w/ instinct & intuition, but this is what should be done.

Let's piece it together in a very ordered and congruent and progressive manner.

We have several ways to collect info. Assemblage & interpretation & assessment of chemical knowledge will be the main problem to affect the final result. Example of data collection will be

1. Observation & intuition do work together, along w/ exploratory leads
2. Solubility testing
3. Indexy reaction
4. UV - VIS in all segments that apply
5. NIR
6. Chromatography
7. Electrochemistry
8. Qualitative testing
9. Historic MSDS lit library
10. Titration

II. Model construction, IR, UV correlation, starch

It does not need to regarded as an end product but a tentative model that gives us somebody to work forward with.

Now ya Could write a paper As is on the function of synth blood & hemoglobin Conformation.

The sufficient try and probably make sense.

The TSP model is its own venture that will be quite an investment and pursuit. To compile that w/ the hemoglobin would be too much for people to handle.

Hemoglobin needs to stand by itself.

Title of paper:

Cross Domain Bacteria (CDB): Synthetic Blood & Hemoglobin

Care: Observation
UV ~~UHR~~ NIR
Catalase
Digital
Kastle Meyer
Historical precedent

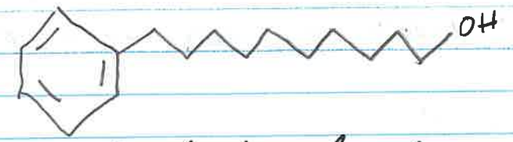
Difference also. Not human blood, synthetic blood w/ hemoglobin. Occurs after polymer matrix.

ChemSketch by ACD looks to be a marvelous program that has been improved on at free rely smoothly into ChemSpider, another marvelous program.

The newest avagadro program is not running on LinB.

Ok, let's see where we go. A starting point on the TSI is that we seem to have an aromatic with an alkyl alcohol. To reduce the solubility, it is suggested (Chemspider) that I will need to get about 10 carbons on the alkyl chain. Last suggestion is therefore:

10-phenyldecan-1-ol



The case for dendritic chemistry seems to have increased a much by the development seems centered on solubility as well.

The other orbs by me will be the incorporation of 1. plasticizer 2 polymerization 3. inorganics

Recall your gas chromatography work where you had extended the carbon chains from pyrolysis work to be on the order of C13 or so as I recall. I think you maxxed out on your capability with the GC and did not know where that ending point was.

We seem to have something very important going on here.

We are getting strong peaked absorbance @ 260nm.

This corresponds to either nucleotides or nucleic acid. Obviously a very big deal.

The is from a scan of 300µl into 3 ml of the H_2SO_4 dissolved TSP-70 sulfate polymer material.

Nucleotides are the building blocks of nucleic acids
Nucleic acids are the products of nucleotide acids.

There are four in DNA:

Thymine and adenine
Guanine and Cytosine.

Now DNA dissolves in water. Our TSP-70 is insoluble in water and also ethanol. Therefore our sample here is not "DNA", so to speak.

But it could be:
1. Nucleotide(s)

2. DNA somehow embedded within a matrix that is insoluble in water. We already know we have an aromatic/alkyl alcohol based structure from last info available.

Then we can go the word and then build up the molecule further.

Nov 06 2023

One thing to notice is that TSP 10 has a large surface area. The most advanced development, i.e.,
1. synth erythracyclines
2. Aromatic + alkyl alcohol
3. Polymer
4. Potential nucleic acids

is taking place on a surface layer. Therefore the larger surface area of the culture be shown itself to be of importance. The culture is ~ 800 ml in a surface area of ~ 7-8" diameter. This means that the layer culture has shown itself to be a distinct advantage, as real growth of the mycotoxin and evolution has not taken place in any of the smaller or petri dish cultures.

It takes about 2-3 weeks total to have such a culture in place so there is a lag time. Also the total culture volume is began with is on the order of 6000 ml (4500 + 1500 ml in two separate containers). These are then condensed and filtered down to ~ 800 ml, so reduction (or concentration) by about 7 times. Concentration also requires several days @ ~ 130° F.

~ 4-5 days growth
~ 4-5 Concentration
~ 10-14 days incubation (40° - 75° F seems to be sufficient)
Surface polymer appeared.

Next topic. We are to repeat the work of yesterday Nucleic acid detection directly by UV but in the polymer matrix is highly significant. Let's see if it verifies.

—

Comments w/ hemoglobin paper.

—

"The principle of the UV absorbance method is that nucleic acids (RNA or DNA) contain conjugated double bonds in their purine and pyrimedene rings that have a specific absorption @ 260 nm)"

ScienceDirect.com Shen

pH can affect absorbance, note we are in strongly SO4.

Overlap of absorbance is from 255 - 262 nm.

---

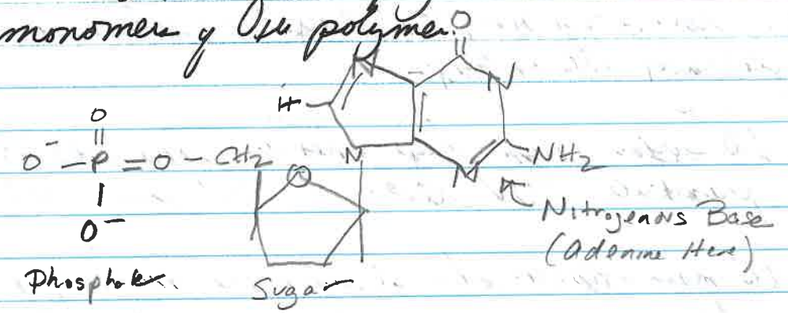
* Confirm absorbance centered @ ~260 nm. Presence of nucleosides (or DNA) shown clearly & to confirm w/ the TSP70 surface polymer.

Sample was 200 ul w/in ~ 3 ml of cuvette
Sample + in setup ~ 50% H_2SO_4

A nucleotide is composed of 3 parts. I am working on getting these into a model form.

1. Deoxyribose
2. Nucleobase (four of them exist, guanine, adenine, cytosine, thymine)
3. Phosphate group.

Notice that DNA itself is a polymer. The nucleotides are the monomers of the polymer.



Structure of a nucleotide.

Recall we also have protein and tyrosine detected by UV. These give us

1. Protein - Tyrosine +(?)
2. Aromatic + alkyl alcohol
3. Nucleotide (Possible DNA)

] all from UV

4. Hemoglobin - direct detection

As constituents of the TSP 10. A big deal here.

5. Erythrocyte - observation - synthetic

We see that we likely have at least five entities defined to exist up in the COB.

Mostly the information comes solely from the TSP 70 surface layer and the use of UV.

There is a lot more ground to cover.

When we look @ TSP after ~ 170 days incubation, we all generally three things:

1. The surface polymer layer that we have started previously UV cleared with (some residual IR).

2. The main solution that occupies the majority of the volume.

3. A whitish settled layer on the bottom.

Let's go back to the majority solution. We know how HCl and the titration can be repeated under more known conditions.

* Our starting pH on the row as of the date is 3.74. There is extraordinarily low and is a harbinger of the damage that can ensue.

This should facilitate to some degree the process of determining $P_i(s)$.

Our last titration was on Oct 30 and I was working on the acidic side. We need to switch to the alkaline side and will use 50:1 NaOH-KOH. Titrate the buffer.

Titration on the spreadsheet.

Amended titration. We have an eq pt @ 13.2 mL
Our pH titre = 5.26
½ 13.2 = 6.6 mL Corresponding pH = 4.31 = Ka = pI
$K_i = 10^{-4.31} = 4.90E-5$

What we see that the solution is cloudy and whitish which indicate that a precipitation has taken place.
Because the pI might change as the age and pH of the TSP10 culture changes. But we do have a data point here.

The use of the data and TSP10 pH.

Nov 07 2023

What I think we are seeing in a titration when the proteins in the culture are likely changing form and concentration as they incubate over time.

This is indicated by

1. Significant pH changes that are taking place in the culture with time. They have ranged 3.75 to 8. At this point I cannot predict what direction it is going but more often than not acidic over time. The worst case of the pH dropping to 3.75 is rather extreme.

2. The pI values they obtained also seem to be showing a wide range and are varying in value.

It will guide a list of more detailed pH monitoring as well as numerous titrations w.r.t. time to see if this is the case.

On the most recent titration, we can see the precipitation take place. The solution has also clarified by settling overnight. Since there is already a layer that is settled on the plexiglass TSP-10 culture (mentioned in Nov 06 notes) the days that we likely do have different proteins that are settling out w.r.t. time, incubation and change in pH. We have seen this before when an iterative titration was performed.

The collection and comparison of these different precipitate (ie proteins) would they all be valuable.

The production of the polymer surface layer - synth cells probably requires all components to be present, which is still the case for the primary culture TSP-70 that is under observation + study for the last two weeks.

We need to come up with a categorization of these solids that are forming. Then you will have 3 solid forms available in the passage of TSP-70:

1. Settled protein layer (presumed) in original TSP-70 incubated over time. It is known that pH has been in a state of change, anticipated from ~4.6 down to ~3.75

2. Surface layer, showing up as nucleotides or nucleic acids via UV.

3. Titration of main solution volume produces another precipitation w/ pI estimated @ 4.31. The mineral layer settles on bottom. There is a second precipitate.

Therefore at this stage alone you have 5 different components available.

1. TSP-70 surface layer (nucleic acids/nucleotides apparent)
2. Main TSP-70 volume solution
3. Main TSP-70 settled layer
4. Titrated TSP solution, now @ pH ~6.6
5. Mineral precipitated layer from titration.

For there you already have synthesized separation in place.

UV is giving us a surprising amount of information for its simplicity.

1. Protein
2. No Protein
3. Charged Protein
4. Specific Amino Acid, ie Tyrosine
5. Nucleic acids / Nucleotides

Use this as a valuable first layer approach, along w/ microscope...

—

I have viewed the enzyme poly mer layer twice now and how much the assessment of nucleotide & nucleic acids present.

Let's compare the two solution forms next: TSP-10 main volume solution and the titrated resulting solution from Nov 06. (pH should be ~ 6.6 vs ~3.75 for TSP-10 incubated main solution volume). 500 ul in 3 ml

—

The culturing process does appear to be most fruitful @ biological temperature, eg ~ 98.6.

—

I used 500 ul of the titrated solution in 3 ml cuvette. Very smooth and strong absorbance curve in UV. Here we have a peak @ ~ 270 nm & 269 nm & have a valley @ 258 nm which would show a lack of separation from the nucleotide mixture.

Bacteriophage visible arrays ...

Let us also make a practice of collecting VIS-NIR data from 700-1100nm to acquire as much info as possible. 700-1100 is always weak signal so we a single carefully calibrated detector.

There is an interesting topic showing up in the UV spectrum. There is/are a pattern of literature speaking of bacteriophages with UV absorbance @ 269 Nm. There is one measured value. One paper also shows a spectrum match ref very closely in the general profile, peak, valley, increase & decrease absorbance as well.

One such paper: ResearchGate.net, Methods in Mol. Biology, Nov 2017, Filamentous Bacteriophage Viruses.

"Filamentous bacteriophages are elongated semi-flexible viruses that infect bacteria". Single strand DNA.

* 4 papers on bacteriophages are showing up under the 269 nm search.

Filamentous bacteriophages are ~6nm in diameter and ~1-2 um long.

"They have shown promise as tools in nanotechnology and immunology".

"Among the simpled living organisms known". Much more diverse and widespread than originally appreciated."

† Isolated and characterized in the early 60's. Many have been implicated in pathogenesis.

Abdesignlabs.com has also a spectrum which matches again very similarly to our profile w/ max. again @ 269 nm (shallow maximum as we have.

Absorption spectrum is based upon the constant relationship between the length of viral DNA and the amount of the major coat protein VIII.

The spectrum shown is also of "filamentous phage".

It certainly looks like you have a good match here. It seems that

1. It would be difficult to match the spectrum otherwise
2. Filamentous is a strong match
3. Bacteriophage - Gram Domain Bacteria is a strong match.
4. Infect almost invariably Gram Negative bacteria

Your chance of matching otherwise & the level are quite low.

5. Make much DNA w/ E Coli
6. Bacterial & Archael 7. Nobel Prize 2018

~~Now let's go to 700-1100 nm.~~ Not yet.

Bacteriophage pathogenesis is a significant topic here.

Dedicated search to:

Filamentous Bacteriophage Pathogenesis
is always insightful. Inoviridae.

* 7 Xmatches 1000 5.

8. Big Im
9. Persistent Infecti-

At the leading edge of biotechnical research e.g.
1. DNA sequencing
2. Gene expression and protein production
3. genetic engineering
4. genome sequencing

"Phages... are the predominant biological entity on Earth" NIH.gov EMBO 2019 Jun Iain Hay

"filamentous phages can be longer than the bacteria they infect."

in 2018, the Nobel Prize in Chemistry was awarded for "advances in directed evolution" involving filamentous phages.

It is a cooperative relationship w/ bacteria

* Biofilm production is supported and enhanced.
* Persistent vs invasive infection by the host can (cannot) can be a feature.

* In essence, we may well have the isolation of a specific filamentous bacteriophage. I have yet to see any circulation or collaboration of the point,
* also nucleic acids or nucleotides seem to be at hand.

With the titrated solution, we have a most
definite peak identified @ ~~963~~ 962 nm.
The is the alkyl alcohol.

The is very sensitive work w/ UV and very low absorbance
values but also very definite if performed carefully

There may be signs of the aromatic C-H also near 714
but as well pass on that for now.

What we see is that as the cuvette settles over time,
the alcohol is going to separate and rise in the
layers of the cuvette. It, in the case, also becomes
stronger and easier to detect w/ the passage of time.
Definite 962 nm corresponds to alkyl alcohol as
these w/ no hydrogen bonding.

Now let's set the pH of the titrated solution.

The pH of the titrated solution is 6.20.

There is via the pH of 3.75 prior to pI determination
and precipitation.

The change is significant and it shows that the
acidity is almost certainly deriving from
the precipitate, primarily protein.

You could do a couple of things here. There is, however, very little precipitate to work with.

1. You could collect the precipitate, re-acidify it and see if protein shows up in UV.

2. You could take the acidified protein, run it through a titration and see how it compares to the first titration/pI determination. (very little to work with).

So what we have from the titrated 20 day TSP-10 is

1. Strong evidence of a bacteriophage in solution.

2. Separation between an acidic component, presumably protein, and the more neutral solution presumably containing a bacteriophage.

Let's centrifuge this to try and make it through the UV unit.

This was very successful. + have more precipitate to work with than I anticipated.

Curiously, there are actually two layers that show up in the precipate.

1. One is pure white
2. The second, greater in volume, is light brown.

So now we have another separation that is taking place. You will need to come up with a coding system.

Culture Codes

Observation: In the 1500 ml culture in place for ~ 48 hrs the filament form on the surface has developed very early in the game, w/ ~8 different colonie forms.

I have transferred them to the main filament colony now developing over the last two months. Every one of the culture is also important to that form of development.

—

I have developed a preliminary culture coding system. They will be sorted and stored on a spreadsheet (Excel sort).

The Code template is now (as an example

TSP 70.1 D
(S)
6.20 1107

TSP 3 LETTER CODE NAME
70.1 CULTURE SERIES. SUBCULTURE
D N=NATIVE P=PRECIPITATE D=DECANT C=CENTRIFUGE
A=ACIDIFIED S=SOLUBILIZED

(S) S=SURFACE, M=MAIN VOLUME OR MIDDLE, B=BOTTOM
(NATIVE CAN BE ALL THREE)

6.20 PH
1107 DATE

Additional pH-Dates can be added and tagged on.

Culture variation are becoming increasingly complex so it is necessary to extend the coding procedure.

Now TSP 70 now identified as TSP10.0N (SMB) 3.75-10/9 has a precipitate layer on it also.

We can collect a small portion of it also, centrifuge as well, and then eventually compare it to.
TSP10.2C (B) ?— 1107 (? means pH unknown) (solid)
It will be named TSP10.5C (B) ?-1107
and save it for comparison to 70.2,

You can now prepare NIR Cards for 10.2 & 10.5
Recall 10.2 has two layer showing up.
For now just need to use the brown (top) layer.

We have so many separations taking place now that it is a challenge to keep track of them. These are actually even a small fraction of what likely exists.

Our two next important separations are
1. Apparent nucleic acids / nucleotides
2. Apparent bacteriophages.

It is surprising how much we are learning from UV. The was unexpected.

I think that we should try to get 10.5 under UV in a soluble form.

Now here's a problem.
10.5 is coming from the bottom settled layer in TSP 10.
But TSP 10 is already @ pH 3.5! Did it precipitate out @ from a hydrolyzed pH of TSP 10. We know that TSP 10 native form should have pH of about 4.5.

We believe we recovered two pI's from the solution one by alkalies and the other acidic. Let's try to find those numbers.

—

Notice our note of Oct 30:
We determined a pI of 4.09 and we made note of how weak the titration curve slope was.

On Nov 07 we run a titration and we get a pI of 4.31. I note also how gradual that titration curve was as well.

Also on Oct 30 I determine two of the pIs are
6.25 & 10.2
and suggest that we do have 3 possible proteins.

—

Now our question is, what was they titrated? on Oct 30?

pI_2 of 10.2 was determined by "TSP from LC."
(see Oct 29)

Titration shows us this is happening

pH Changes: Significant changes take place

The involved the combined vials of #3, 9, 5P from an LC run. The LC run was from TSP w/ sodium Carbonate that caused a precipitation. We used a decanty that on LC

—

I am now looking @ a comparison NIR plot between TO.5 (Native Precipitate, ~200 days) & TO.2 (Titrated precipitate)

TSP TO.5:

1195 (TSP TO.5)
Methyl (1195, 1194)

TSP TO.2:

933 (TSP TO.2)
Methylene (932)

What I see here is a shift from the methyl groups of TSP TO.5 (Native) to a methylene form in TSP TO.2 so aliphatic chains are being created as a result of titration. They're further evidence of a disturbance to methyl groups that stand as the pH is raised during titration.

1506 (TSP TO.5)
Aromatic Amine (1502.5)
NH Amide / Protein (1510)

1515 (TSP TO.2
Polyamide (1515)

The ways we have a shift in polymerization here are as a result of titration. What the media is that we have an increase in polymerization of a protein / polymer w/ an increase in pH to an alkaline pH.

1582 (TSP TO.5)
Alkyl Alcohol (1583)

Rather clear here a shift from the alkyl alcohol into vinyl with the raise in pH

1611-1643 (TSP TO.2) Broad
Vinyl, Vinylidene
Vinyl (1613)
Polyamide (1618)
Acrylate (1621)
Vinyl (1621)
Vinyl (1630)
Vinylidene (1631)
Vinyl (1635)
Vinyl (1637)

Nov 08 2023

From the NIR run & study last night, you have a little awareness now how spectroscopy can help you. Beside Compositional info, spectroscopy can be used to determine changes in state, i.e, reactions that are taking place. This could become quite powerful.

You are now aware that your cultures are in a constant state of change:

1. Nutrient levels
2. Temperature
3. pH

are all affecting the culture on a continuous basis.

By determining the changes in state you will get a better understanding of what is actually involved and how the combinations and reactions are taking place. all modes are going to be important.

1. UV 230-300 nm
2. VIS-NIR 700-1100 nm
3. NIR 900-1700

They each have somewhat very important to offer.

The large primary cultures that are now to held intact require a monitoring system also for the above temp, pH and data log: start.

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I have a method of improving the filtering rate for the cultures, instead of a coffee paper filter. A reusable fine mesh coffee filter in a squeeze, much faster w/ less waste & equal results.

Do one of the things you would like to do now is to start to monitor changes in the culture. Two ways to assess, therefore:

1. Composition
2. Change in culture, assess reactions, etc

There are so many manipulations that are taking place that you need to get a handle on general components and major changes that take place.

You buy cells now as nucleic acids & bacteriophages. Cells and how are they coming into being?

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Blood inspection day. It came out fairly well & maintaining clarity COB and fairly modest roulleaux, decent glomerly.

You do see that slide preparation does affect the roulleaux presence on the slide, however. I used by moderate pressure & low angle.

You need a film of blood, not a streak. Ideally even in texture. Need two slides, not one. Find max stain alone density to evaluate. Avoid edges.

The exercise of ~ 6-8 slides was hemocue. Slide prep can skew the interpretation.

We need to systematically approach the culture evolution and seek a generalized description of this evolution.

What should be measured in a sequential fashion?
Start in elementary fashion.

1. start
1. pH, TDS, specific gravity @ top
tells you nothing to little of what is acting or reacting

2. UV 230–300
UV 700–1100
Starts to tell you a little bit. } Coded

3 NIR Card

1st trial of the method. Data will now be stored in a spreadsheet.
Culture range is TSP 100.1(N) 3.81–1108 300ml used.

24 hrs - There is no love a protein @ the onset of the developed culture

Now recall when we measure 700–1100 nm we have a very sensitive measurement. You must use the same cuvette as the reference, scale accordingly, and allow adequate time for stabilization. This means multiple uses

We do have a different plot @ 691nm. 961nm.
There is our alkyl alcohol.

The means therefore that we have protein and alkyl alcohol @ the onset of the culture.

Can these be separated by chromatography?
Can these be separated by pH?
What about NIR readout?
What about electrochemistry?
They obviously would all be of interest.
* What happens when you put it on blood?
Vit B impact?

Here is a big question. Does an exogen enzyme immediately have impact? Answer is yes.

You have perfection of results. You basically can easily show that you have interplay with the fundamental protein formation as soon as it is formed.

This is monumental. I would only need a very weak enzyme solution to produce the effect. Three plots:
0. Water reference
1. TSP 100 Complex in general immediately upon formation (~280 absorbance)
2. TSP 100 + Enzyme - a very different profile - Conclusion 40ul protein interference
3. 40ul Enzyme alone - Can even different profile which is required & Concluded that reaction take place.

What this actually means is that anybody that comes after the formation of the protein, which is encrypted by complex biology, has been nullified as a primary form.

You can study the other aspects to no end and you most likely will, but theoretically there is no requirement to do so.

Interference w/ the protein formation & the onset of delivery or regulation was the "prime directive".

The case has now been proven on multiple occasions, on the one most clearly and emphatically w/ the proper controls.

The native TSP (TSP 100.1) has no obvious structural impact when mixed w/ human blood.

& have started a comparison culture w/ C. Enzyme designated TSP 100.2

1. Would like to start filtration of TSP 100
2. Run NIR of TSP 100.1

Nov 09 2020

The culture log is already showing itself to be unique, the ability to sort in the spreadsheet is making interpretation easier.

already an interesting change. The enzyme culture (TSP101.2) is showing some change in addition to the functional structural information strongly confirmed w/ UV spectroscopy.

1. pH goes up slightly from reference (Recall 40 mg in ~10 ml) +2%
2. TDS goes down ~10%
3. Specific gravity, ie PPT, increases 10%

The interesting we see measurable change in all reports measured. The UV is especially important - structural change.

40E-3 gms / 150 gms = x / 70E3 kg x = 16.7 gms (.7% H_2O) = 14.6 gms ≈ 15 gms
Equivalent body weight.
i 3 gms per use.

Ok, let's start the titration to the alkaline side.

Before then, let's run a centrifugation trial on TSP 70 (Middle) and the next increment culture TSP100
1. Visual — nothing indicated here.
2. Specific gravity — Will not continue here yet.
3. UV

Well, the titration produced an assay result.

The primary culture fam, now TSP 100.0, at the beginning of the full cycle produce a protein of a buffering capacity that in the blood.

Exactly @ biological pH, I error of instrument ($0^{+}-0^{2}$ pH) the TSP culture buffer extremely well, as blood would also do. — Quite phenomenal to see.

Now let's look @ the pI:

Eg: pt in on the order of 21 mL.
1/2 eq = 13.5 mL pH corresponds is 5.33
= pI.

The isoelectric point of serum albumen is pH 4.7

The pI of human hemoglobin is lowered about 1 pH unit [(?)] @ the air/water interface.

Conclusion - definitely a systematic blood type protein is inherent in the culture at the onset of creation.

The reason the pH and it does not require 2 weeks to develop, the protein is already buffering in a large and as human blood does.

" Theoretical calculation of pI can easily give an error of 0.2 pI or larger."

And guess what?: The greatest number of proteins in the blood have a pI of 5.3

* Can't get a better match than this
Our protein under analysis

* 1. Matches human blood proteome distribution
max of pI = 5.3

* 2. Our protein has buffering behavior identical to that of human blood in the same range as human blood does.

We definitely have a match for a synthetic blood, w/out any doubt whatsoever.

So our set of matches is:

1. Viscosity, geometry, biconcave, rigid

2. Hemoglobin

3. pI match w/ human blood proteins

4. Blood buffering capability matched.

Nov 10 2023

Four topics (ie, discoveries) have emerged:

1. Blood pI vs synth blood pI
2. Synth blood buffering capabilities
3. Nucleic acid presence
4. Bacteriophage presence

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It is of interest that the chromatography column solidified a week + has turned greenish in color. This would seem most likely due to an Fe^{+2} complex/ion that has eventually separated from the column residue.

Today:

1. File reviewed - PDF
2. Lab notebook uploaded
3. TSP 100 monitored, spare versions labeled
4. TSP 100.2 monitored

5. There is now an important pI precipitated TSP version to evaluate.

Some important learnings @ the onset of a developed culture:

1. NIR work says that a protein and an alkyl alcohol are immediately available.

2. The protein, during PI analysis, precipitates out during titration and titration results data indicate we have a material w/ both buffering characteristics as well as the human blood proteome.

We do not yet know the difference between the remaining solution and the precipitate, but we surmise that the precipitate should interest the synth blood characteristics.

3. The L enzyme is showing an impact, apparently across the board, on the TSP culture development @ the onset. The include most especially UV, but pH, TDS, and PPT (refraction) also indicate change. All of this, if the case, will be highly beneficial.

Let's start by monitoring this, TSP 100.2.

Here is what is learned from the fundamental monitors, ie, pH, TSD, PPT (refraction)

The enzyme culture is reducing the amount of dissolved materials in the culture. In other words we are causing some type of conglomeration or aggregation of materials. * This is taken as a variable. Any change in UV, TDS, pH, or PPT indicate a disruption to the protein.

The pH is holding constant.
Now let's look @ UV.

The process is very valuable. In the first time you are getting progressive logical data and feedback on the dynamic behavior of the culture w.r.t. to time.

At the time, and undoubtedly with great emphasis in the future, the dynamic behavior also include the impact from enzymes.

You want the monitory system to include:

- pH
- TDS
- PAR
- UV
- NIR

} as a minimum. Maybe MID IR will come back into the picture? Definitely a powerdome

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Looking over monitoring computation now fully in play, we see that 100.2 should be eliminated. This is because it was created using the delute culture. It is not representative of the primary concentrated culture under use. Therefore we want to transition any enzyme comparisons to the concentrated culture, which remains at TSP 100.0 and will supersede any of the enzyme comparisons.

The only thing that can be said of enzyme certainty is

1. The L enzyme structurally changes the protein via UV examination
2. It may be decreasing the TDS as a function of time. More data is needed.

Health Notes:

Elevate the urine pH. Seek ~7.0 if possible
(high end of normal range)

Keep body temperature > 97.5 if @ all possible.

Low urine pH & low body temperature are conditions of susceptibility.

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Ot cultures are fully anticipated relative to the concentrated form of TSP100.0

TSP100.2 is helpful in preliminary sense. I will look @ UV profile. Eventually TSP100.2 (NOT CONCENTRATED) will be deaggregated. discarded in favor of TSP100.3 (Concentrated w/ Enzyme). TSP100.4 will be a control: Concentrated w/ no Enzyme added).

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The health list is now:

1. Enzyme benefit
2. Vit B – evidence based
3. Ultrasound - polymer impact
4. Balm – botany act
5. Salt - electrolytes
6. Body temp > 97.5
7. Urine pH on upper end ~7.0 (Urine buffer)
~~and the usual~~
8. B the usual candidates, antioxidants, etc
9. Skin excretion as a major factor - indicator

Preliminary Enzyme feedback:

Here is what I see. Although preliminary the enzyme is having an impact upon the culture development. In UV, the TSP100.0 peak is shifting from ~275nm to 265nm. In addition, the UV profile is different w/ an increase in absorbance also Lady plant in the 258-259 Valley of TSP 100.0

The means beneficial. Definite structural impact is occurring.

Now let's look @ VIS-NIR. Recall that you must use the same cuvette. Sensitive work.

Our region of interest will be 880-1020nm.

General Comment: Here is something that must be happening w/ the alkyl alcohol. It must be in a soluble form, a mostly soluble form @ the onset of the culture.

But then when you analyzed the solubility of the synth cell-polymer, you found that an alkyl alcohol treated there via the NIR Card of the polymer that was made. But here it was very insoluble.

* The mean that the length of the carbon chain of the
* alkyl alcohol is increasing as the polymer matrix
* develops in the culture. That makes a lot of sense.

As expected, we have a very sharp defined peak, although weak in TSP 100.0 (primary Concentrated culture) @ 962 nm corresponding to the alkyl alcohol. At this stage, we know, however, that it is a short chain alkyl alcohol because of its considered H_2O solubility. We know that it forms a separate layer in the cuvette over time.

We now also see a 930 nm peak which is methylene, which also makes sense for the alkyl alcohol. This only showed up on time in the 5 second sweep, so it will be disregarded.

Ok, what we see is that the existence of the alkyl alcohol is not altered in any way w/ the use of the enzyme.

Therefore:

1. The protein (on total) (w/ pI 5.3) is affected by the enzyme.
2. The alkyl alcohol is not w/in 72 hrs.

Next, prepare Nile Red for generating.

Set up for 100.0 vs 100.2

Now let's start thinking about how to approach the state of the solution (i.e. TSP 100) that has now been titrated to determine the pI.

So here is the sequence.

TSP 100.0 @ the test was not yet Concentrated
This was titrated to determine the pI, which results
@ 5.33. The solution was originally quite acidic
@ pH 3.01.

As a result of titration, we now have two components.
A solution (~95%) and a precipitate settled
on the bottom (~5%).

Let's separate these two components.

Designate as TSP 100.5 (solution) + TSP 100.6 (precipitate)

Nov 12 2023 - Veterans Day

It is going to be very important to adopt the method of dynamic evaluation of the TSP Protein complex behavior. Step by step to see the change w.r.t. time

I now have a NIR plot comparing TSP100.2 to TSP100.0
This is L. enzyme VS pro L. enzyme.
The signals are weak but we will work with what we have.
There is not a massive change in the NIR profile, but there is a difference and it is hopefully to become very important.

The first item being watched closely is 960nm for the alkyl alcohol behavior. The signal is extremely weak but can it also in the VIS-NIR spectroscope. There may be a slight decrease of absorbance in this NIR region but it a too weak to draw any conclusions or even inferences yet. So I will keep an eye.

The profile pretty much match and coincide the per all the way across from 900nm to 1590nm.

However above 1590nm there is a sharp and completely cut off absorbance while the native TSP 100.0 remains in state of high absorbance. So let's see what this corresponds to.

Well as hoped for, the there we can observe the polyamide (1598, 1618nm), the vinyl formations (1613, 1621, 1630, 1637), aliphatic hydrocarbons (1631, 1635) and halogenation (1655-1661)

There is, therefore, extremely promising. I shall keep a close eye.

Now what we continue to have matches with are

~1185 Methyl

~1384 Methyl

~1543 Secondary Amine

~1592 Alkyl alcohol or Polyamide

This is exactly what we are trying to learn. The enzyme may indeed be interfering w/ the protein/polymer formation in any significant ways.

Monitoring of cultures is being recorded.
Body temp and urine pH favorable today. Feel better accordingly. Strong indicator of poor health w/ low body temp and low urine pH now TWICE.
Strong sleep pattern of correlation w/ health.

An observation on 100.5. This is old count from titration w/ a pH of 7.29. Now a totally different chemical.
There is slow surface growth on the two vials of same sample. Viewed under scope.

Now there is a very curious observation on TSP100.5 (Decant after titration) on the surface layer

3-4 entities are observed

1. COB colonies
2. Polymer - COB matrix undergoing formation
3. Single synthetic cell observed - may disconnect
4. A grid like development within the polymer formation that is exactly in the same focal plane. It does not appear to be a slide or cover slip artifact. The rectangular grid development seems to occur in direct proportion to the density of the polymer matrix. Try to keep one of the vials undisturbed to be able to monitor the development.

Crystals were anticipated. None are seen. It appears the polymer development initiated.

We now know if we bring the native culture up to a pH of ~7.29 that this is likely to occur on the decant alone. Eventually I would hope to be able to gather enough of the material for NIR analysis. UV uncertain since it is by solid form.

Let's pick up the "vitals" of TSP100.5 pH, TDS, & PPT

In one section of the slide, the COB-polymer matrix has aligned itself as a major grid element.

In addition, the rectangular grid DOES NOT appear where the polymer matrix is not developed. These facts indicate that the grid is quite real.

On the vitals, what we see w/ TSP 100.5 (decant of tibrate) is a significant jump in TDS. On the order of +40% after 24 hrs. pH & PPT remain ~ identical.

The shows that the three (3) measurements are independent of one another and this is good. Definitely do NOT assume that TDS & PPT (specific gravity) will follow in tandem w/ one another, although it might seem that they would. They can, as the shows, be quite different from one another.

Now I would like to get 100.5 into UV. The can not yet involve the surface layer material; there is not enough to work with.

What UV will indicate here (and NIR) are the conditions, along w/ the vitals, that are conducive or supportive of the surface layer development (i.e., polymer—CDB matrix, it appears).

The sort feature of the spreadsheet vitals is especially useful. Culture subgroups can be assessed much more easily now & show flipping through all notes. Vitals instantly comparable.

TSP 100.5 ± 24hrs

TSP 100.5 Significant TDS change from ref TSP 100.0 (tibrate decant)

TSP 100.4 No major change (Control for Enzyme Study) w/ TSP 100.0 No

TSP 100.3 shows some enzyme influence w/ TDS & PPT on the order of ± 10%. NIR shows this also. UV shows it also.

TSP100.2 is to fade away and be discarded.
The use is a culture prior to concentration. Done.

So in summary again:

1. TPS100.5 shows major change (expected due to filtration) in all vitals from TSP100.0 reference.

TPS100.5 shows internal significant TDS change w/in 24 hrs. This is decant from filtration.

2. TPS100.4 shows an ~ +20% in PPT relative to my TSP100.0
TPS100.4 is a low volume version (~150 ml) of TSP100.0 also w/ no enzyme added to use as control. The primary purpose of TPS100.4 is to study how the surface area of the culture affects surface polymer - biofilm cell development that took place w/in the 1500 ml culture of TSP 0TP. This indicate a difference may well occur.

3. TSP 100.3 shows modest change in both TDS & PPT, from the rest TSP 100.0 culture on the order of +/- 8% and +12% respectively. TSP100.3 is the culture w/ 40 mg of L enzyme added. We know that significant change is occurring as reflected in UV & NIR analysis. A very important culture. UV shows alteration of the protein structure. NIR shows a major effect upon the "vinyl" groups, i.e, decrease in the reflected in a use of the non concentrated version (i.e. TSP100.2)

Now the focus today shifts to TSP 100.5, the decant by filtration. The precipitate from filtration TSP100.6 will come to the appropriate time after each.

What we do know is that the first filtration produces an important separation in which a soluble and a precipitate form. We are setting up an presumption that the precipitate should be a primary synth cell protein because of the pI makeup to the blood proteome determined from filtration.

Now the only first interest is on the soluble portion of filtration, namely TSP100.5.

OK, here is what we have.

TSP100.5, the filtrant decant, still contains protein, however, there is when the 272 nm peak shows up, indicating that we have a protein strongly based upon tyrosine.

The protein is therefore quite different from the first protein, that from filtrate. Buffers and pI results show that it is a synth blood form. We also know that protein is insoluble @ pH ~ 7.29 and the current one in TSP 100.5 remains highly soluble and appears to be tyrosine based.

Now, in addition to the vitals we see that it has become important to develop a spreadsheet, possibly a database in the case, that helps us to sort out the organic features of each culture. That would be good and go better than a spreadsheet.

The database project will be quite involved but also very helpful.

Ok, we have a database in place. We will now build the database up as we learn more and/or integrate additional accumulated data.

Ok, the database will start to fill out as we work through the culture dynamics.

We now have

1. Activity Database
   1. Topic 2. Sample 3. Description 4. Date 5. Volume
   6. UV Absorbance Peaks
   7. NIR Absorbance Peaks
   8. pI determinations
   9. Free form Notes

2. Spreadsheets for Culture Dynamics & Coding

3. Titration & Charting & Pi Determination Spreadsheets

The work is far too complex and difficult (if not impossible) to analyze w/out these assets

100.5 has no significant VIS-NIR absorbance 700-1100nm

100.6 does have the alkyl alcohol @ 962nm

Acidifying the precipitate is an effective way, depending upon pH behavior, to get a UV spectrum of a solid

Nov 13 2023

TSP 100.5 does look to have a surface layer. Indication are that it is of a polymer nature. Use of titrant decant. Will need to make more of the w/ a larger surface area. Currently legible for microscopy but not UV and NIR

We have an NIR comparison between 100.5 & 100.6
100.5 is titration decant; 100.6 is titration precipitate
There are two items that are discerned from it, although signals are very weak:

1. 100.6 (Precipitate) has increased absorbance @ 915nm & 1643nm
915 is Methyl
1643 is Vinyl (1637)
A great deal of vinyl occurs from 1620-1640.
Our 100.6 shows elevated absorptivity and confinement of absorbance in the region.

Another thing we seem to see here is a shift from the amide/protein nature of the decant to this vinyl region.

Let's see what UV said on 100.5 regarding protein.
Yes, indeed. 100.5 shows protein/tyrosine signatures @ 272nm.

- The water is consistent.

Culture Dynamics w/ 1st Titration

The there important. We have an entire series of events here that act successively.

1. We start w/ TSP 100.0 Native culture result.
2. We titrate it. Blood pI and blood osmolar buffering capacity registered in the titration process.
3. We are then left w/ a solution (TSP 100.5) and a precipitate (TSP 100.6)
4. Analyze Analyze of the solution both by UV & NIR say that we still have another protein in solution. The protein determined to be of tyrosine nature.
5. On the same solution we prepare we see a polymer forming, verified under scope. Not enough material for UV or NIR analyses on it yet.
6. Then we have a precipitate. The precipitate shows the existence of:
   1. Vinyl (NIR)
   2. Methyl (NIR)
   3. alkyl alcohol (UV)

Therefore there is saying that we have a desired polymer form appearing.

There is quite a handful to be working w/ already.

Therefore:

1. Need more volume and surface area developed for 100.5
   Doesn't appear recycle material polymerizes
   UV adds NIR works.

2. We apparently now have an additional protein in
   solution in 100.5. Need to attempt pI determination
   here. Known to have tyrosine.

3. We have our first separation of a single component
   as well as the alkyl alcohol in 100.6
   (precipitate). Apparently 100.5 (decant) has
   neither of these but still contains another
   protein.

4. Remember also that our precipitate of 100.6 actually
   has two layers (grey and white) and no attempt
   has been made to separate them yet.

Need to develop the plan to approach the situation

Observation: The spare / backup samples of TSP100
(~ 500 ml each) are showing some separation
of the layers now. Plot to go the main 1500 ml
~~1000 ml~~ volumes of TSP100.60.

We also have our enzyme monitors to work with...

We want to progressively also work toward replicates of the nucleic acid, bacteriophage findings. Like we all done w/ the mature culture of TSP 70 which still exists in plenty of supply.

Next & monitor enzyme & control culture vitals
Recall 3 main Computer assists exist now:
1. Titration spreadsheet, graph, derivative
2. Culture logs - dynamic record of vitals
3. Lab database
4. Chromatography spreadsheet - monitor

TSP 100.5 is showing some strong fluctuations (± 30-40%) in both pH and TDS. PPT (specific gravity) is perfectly constant.

TSP 100.3 & 100.4 is the enzyme influence - control pair 100.3 has enzyme, 100.4 has no enzyme.
1. pH difference is slight
2. TDS difference is ~ 10%
3. PPT (SG) is ~ 2.0%

Any difference between these cultures is to be paid close attention to. Signs remain encouraging here.

Let's Compare UV spectrums of these two & VIS NIR

We looked @ a preview of the culture (via 100.2, now to be discarded)
Compare it to TSP100.0 and note
1. A protein shift from 275 to 265nm UV
2. UV structured shift
3. Suspected TDS shift.

The suspected TDS shift does contain and be confirmed (~10°)
but now we add on PPT shift. Both 100.0 & 100.2
show the alkyl alcohol.

Now let's continue the examination w/ TSP100.3 since it is
in the process of superseding 100.2.

Alkyl alcohol @ 962 remains in 100.3 as in 100.2

There results are by my count no matter how it turns out.
For UV, there is a difference in the profiles between
100.3 (enzyme) and 100.0 (no enzyme) but it is
not overly dramatic.

In 100.0 our strong peak is @ 274nm. (no enzyme)
In 100.3 the strong peak is @ 270nm.

So we do have a shift but it is not dramatic.
We have a shift of the profile of 100.3 to the left of
100.0 ~ 4 ~~nm~~. nm.

There also is some flattening in the profile ~315nm
but this is very subtle.

Well, I hate to say that a phenomenal urine observation is in place. We can but hope that it is a waste product and that a process of removal is in place. Better hope so.

It appeared that some filament structure existed in the urine (but not many but visible). I did capture a perfect specimen for the microscope. Definitely not representative of the urine but it does exist.

It is a massive collection of blood cells embedded into a polymer matrix. It is a very dense collection of a massive number of blood cells. These cells are completely saturated w/ the COB. It is quite profound. Blood is not at all supposed to be in the urine, but there is not just blood and it imparts no color to the urine. It is definitely being contained w/in a white polymer matrix. It does not have a filamentous nature. It is uniform adherence of cells together within the polymer mass.

If it is waste removal, very very, cool damaged cell removal is in place. If not removal, and characteristic growth in the body, it represents a major issue. My take at this point is that it is removal because general blood observations seem to be quite improved and certainly not of the nature. It is a profound observation.

What is being done now is to increase the volume and surface area of TSP 100.5, namely the decant of raising the pH of TSP100.0 to ~7.29.

This will be a new culture to be distributed into petri dishes. It will be designated TSP100.7 D (M) 7.35-1112
Notice that you do use essentially biological pH. Equivalent to titration however here you just need to get the pH raised. Centrifuge will increase rate of separation.

TSP100.8 is mixed volume (~30 ml) of decant & precipitate to serve as a monitor and reference solution for TSP 100.7 Petri dish series (4).

Some projects on tap:

1. Can we capture another surface polymer from 100.5? If so, does the grid structure appear again w/ the polymer development.?

2. Another series of 100.5 needs to be developed for 2nd pI investigation

3. Replication efforts w/ the mature culture TSP 70.

I have improved the microscopy. The surface polymer has a structure down to 0.25 um = 250 nm. It is extremely granular.

Reexamenation of 100.5 surface layer

1. No greeby residue
2. Extremely dense granular nature
3. Cell structure is smaller than COB, measuring @ 0.25 micron - 250 nanometer
4. It would seem that the core of the polymer is the same spherical in shape.
5. Amazing it can measure fairly well down to the size

Well guess what:

Vinyl Nano and Microsphere

Emulsion polymerization provide particle of ~50 - 200 nm in diameter. Emulsifier free emulsion polymerization produce particle of about 100 - 1000 nm. Dispersion polymerization give particle in the region of 0.3 - 10 micron

If you can prove vinyl you have something very important here - Polymer production

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Here is an interesting observation: 100.5 in the titration cell cant and it is perfectly transparent upon production and amber in color. You can tell this by the current monitor, which is 100.8.

However, 100.5, which is now 2 days old is no longer clear! It is cloudy white. So clearly it is remains active. We know that it is producing a surface polymer, however, the polymer may be thrown out of solution.

Yes, the polymer is being formed through out solution this implies that we are going to have a water soluble (partially?) protein.

Another reason for dynamic monitoring...

I can see looking ahead that TSP 70.10 is producing the synthetic cell polymer network on the surface. There is a 3 40 ml Erlenmeyer so this terminates the idea that a large surface area is going to be required to produce this. The well mat things must accommodate future with more trial variations now becoming possible

Nov 13 2023

More and more of the information will be diverted to the database that I have created. The events and relationships are too difficult to identify and locate in a chronological sense because of the activity and complexity level. The database allows me to search and sort to identify patterns.

A general log will always be kept here in parallel but many of the specifics and numbers will now go into the database.

Some observations:

100.5 is showing a steady decrease in pH over 3 days.
100.5 also becomes cloudy over the three days.
100.5 Possibly a sharp temporary spike in TDS 24 hrs in. PPT constant throughout.

100.4 Control culture w/ no enzyme added
1. Shows no unusual pH change
2. Shows a gradual steady increase in TDS.
3. Shows a fairly stable PPT (specific Gravity SG)

100.3 Enzyme addition:
1. pH rather steady
2. Mild increase in TDS
3. Very steady PPT

Thursday 100.5 (Decant, filtration) in the culture showing the most visible change thus far in the vitals.
Appearance of culture has also changed from clear to cloudy, pH has steadily decreased. Strong signs of polymer formation under scope.

100.5 NIR maya plates shows a slight y absorbance from the ~~1511~~ 1610 nm region & the ~~1410~~ 1510 nm region.

It also looks like 100.5 shows a relative increase in the 1420 region.

1610
1510 is in the Amide area.
1510 is the amide/protein area.

1420 could be in the aromatic region, either ArCH or ArOH.

1. Need to review addition NIR plots
2. PE investigation decant 100.0
3. UV protein investigation of 100.5

Nov 14 2023

I am headed into the database (lab log) more and more frequently now. My notes will become searchable and sortable (however, for me, & not the public).

Ok, the will be able to be exported to a CSV file which can then be imported into a spreadsheet. It will thereby be sortable by date & subject.

Only a subset of all fields are likely to be exported for general viewing, ie

1. Date
2. Subject
3. Notes

But the format/subset will be more understandable. Non specific data, esp UV, VIS, NIR, PI, etc data will be easily accessible to me.

The is a feasible system. It can also be an avenue to index by the lab notebook of the future.

It will need to be exported and backed up on a frequent basis or as usual, it becomes quite vulnerable.

Need to work out the reference for the various cultures:

Reference	Culture to the Reference
100.0 Native TSP Concentrate	100.0 Native TSP
	~~100.4 Watering 100.0~~
	~~100.7~~ "
100.4 No Enzyme	100.3 Enzyme — 100.4 No Enzyme
	100.2 Dilute Enzyme
100.5 Decant	100.8 New Decant
	100.9 Mixed Monitor
	100.5 Decant
	100.7 in Petri Dish Serum No Reference
	100.6 is Solid - No Reference

It is actually quite complex to manage the numerous variation of culture, keeping track of the various reference, and complete changes depends upon those variable references.

Right now the information is only within the spreadsheet, I see the hope of integrating it w/ the database @ the time.

But now that we do have it organized, we need to look for any patterns!

It looks like the slope, ie % change per day from the reference will be the most useful indicator of importance, @ least w.r.t. pH, TDS, & PPT

Take them on at a time.
100B is a decant – Ryerson is 100.5 Decant
(young)

Max process activities / Slope of change is starting
to give us more information.

Now in the ideal world I would like to turn
this slope into a probability and then combine
them.

My probability original variance math from
yesterday on part is:

$C = \left(\frac{1}{\text{Range}}\right) \tan\left(\frac{\text{ProbLevel} * \pi}{200}\right)$

if Range = 17.2
$Pr = 95\%$

Math is already
set up in radians
No need to
convert
anything.

$C = .0015$ $.00168 = .0017$

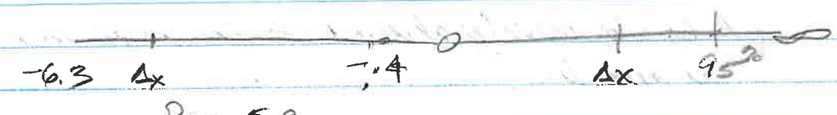
$Pr = \frac{200}{\pi} \cdot \tan^{-1}(C \cdot \Delta X)$ $\frac{\tan Pr \cdot \pi}{200} = \tan^{-1}(C \cdot \Delta X)$

$(\text{Cor}(\Delta X_2)\ \tan^{-1}$ $C \cdot \Delta X = \tan\left(\frac{Pr \cdot \pi}{200}\right)$

$C = \frac{1}{\text{Range}} * \tan\left(\frac{Pr \cdot \pi}{200}\right)$ $C = \frac{1}{\Delta X} \tan\left(\frac{Pr \cdot \pi}{200}\right)$
(95%)

Ok, I have recovered the original probability model work on tail.

Now to deal with a negative range



Range = 5.9

Center the data

$\Delta x - (-.4) = \Delta x + .4$

555 2 $\left(\frac{5.75}{3}\right)^{1/2}$ —

I have established a method of statistically ranking the "interest level" of a culture based upon the "vital" monitoring. It is too complex to discuss/describe here but it is based upon probability research of many many years ago.

One thing new here was defining an alternative range for a given Probability level. I would often use 95% for a given sample set but these somewhat arbitrary depends upon the size of the sample. I have employed only the RMS value (which handles the negative numbers quite gracefully) at equality & a 71% level confidence. Also the final rankings are transformed to flat value by the factor 1.414. The process and solution here is quite graceful and elegant - the took a lot of work to develop.

But the value of the r that it provides me a logical consistent "interest level" score for any Culture that can be monitored for pH, TDS, PPT.

I could develop alternative for solid materials if need be.

Here are my current scores - they would change some w/ time:

Rank				Reference Culture
1	*	TSP	100.8	95%	100.5
2	*		100.9	92%	100.5
3	*		100.5	62%	100.5
			100.0	17%	100.0
			100.4	23%	100.4
			100.3	12%	100.4
4	*		100.2	38%	100.4

These are helpful as it tells when to focus efforts w/ regard to existing culture.

100.8 & 100.9 & 100.5 are all based on decants from titration

100.2 is based upon enzyme addition to a dilute native TSP culture.

Nov 15 2023

See database for lab log.

I have a superb NIR plot (high magnitude, very smooth) of the polymer that I have been able to form by combination of TSP100.5 and borax. Very well defined plateau / no ambiguity. Designated as TSP 100.10

983(2) OH from water (maybe) 979
Phenolic OH (990)

We can and should verify and isolate the H_2O question. It is possible but the issue can be separated.

1170(2) Alkene, polyenes (1170)

1347(2) Methyl (1360)

1436(3) Aromatic Amine (1432)
Methylene (1440)

1480(3) Polyamide (1480)
Secondary Amine (1481)
Amide / Protein (1483)

High absorbance from 1436–1480
Aromatic Amine
is strong here

Valley @ ~ 1572 Polyamide, Amide

1669(2) Aromatic CH (1671)

A polymerized protein is my interpretation @ the point. It is not supported as an alcohol. Phenol – H_2O question is important to resolve.

Nov 16 2023

See database - digital log.

Nov 17

See database - digital log.

Nov 18 - 25

See database - digital log.