CI LABORATORY NOTEBOOK VOL XXXII

Sep 13 2023

What I am up to today is enzyme testing.
I now have ox-bile - a strong candidate as
well as an adequate supply of the L. enzyme.

Cultures are also proceeding very well at double the rate
of production w/ improved temperature control &
incubation.

Am running trials from scratch w/ the latest TSP03.
Peak @ 273.5 shows up. Tyrosine again.
100 ul used. Let's boost to ~~200~~ ul. 400 ul

TSP03 is giving some variable peaks between
264-274 apparently depending upon concentration
100-400 ul in 3 ml cuvette.

A representative curve @ 400 ul is very smooth w/
a broad gradual peak @ ~ 269 nm.
There is no defined peak in the 230 nm range.

OK, the enzyme treated TSP03, now 24 hrs,
is completely different than TSP03, w/ a
very strong well defined peak @ 265.5 nm.
Totally different.

The results look identical to those of Exp 12 collected shortly after treatment by the L enzyme. We have saved that file so we can compare.

Everything says that TSP03 has been altered in a very significant fashion by the L. enzyme. This is of course quite fantastic news.

Bear in mind that we know that the enzyme concentration is quite weak here, which is also very favorable news.

Because I know the enzyme concentration was way too high in the first sample prep it was massively diluted (estimate by factor of 500) correctly almost entirely of TSP03.

I think our case is proven to the strongest now. Recall we have 3 levels of confirmation

1. UV profile completely different
2. No crystal formation under scope
3. No filament production of in enzyme heated vial.

What we now do, beyond repetition and monitoring TSP03 crystal formation, is to see if we can improve upon the general "L-enzyme" effect & that of a specific enzyme.

I have acquired Ox-bile in the present. Enzymes are relatively expensive, so the isolation may not be complete for some time... But Ox-bile is just bench based upon historic culturing methods - see laboratory notes w/ Ox bile usage.

The is, of course, pretty very interesting. Ox bile does not, at least in the short term incubation, seem to be making much difference w/ the TSP03. It is making some difference in UV, but not dramatic. The means:

1. Time is allowed
2. We repeat the entire process over & over w/ the L-enzyme onward, until we get absolutely stable and repeatable results.

There is apparently some folding impact from ox-bile as we do have the appearance of a melt peak ~230, but it is not dramatic.

Let's repeat the L enzyme from absolute water.
400 ul TSP 03 in 3 ml cuvette
300 ul TSP 03 + L. enzyme
Once again 300 ul is too high

There is actually good news since we know that it is having a very dramatic effect.
Now down to 100 ul.
Still high. Drop to 50 ul

Ok, we have the dye and peak @ 265 nm.
We have a positive result. Need to balance the concentration further. 30 ul will still be to much though. 50

There is once again saying very little enzyme is having a large effect. 50 ul is very sufficient in 3 ml cuvette

I have now proven the case beyond any doubt.
Now [redacted] Enzyme disrupts TSP03 in a
very specific fashion. A major
accomplishment & discovery. Which
particular enzyme(s) [redacted] most important
are not known @ the time, but it
does not appear to be ox-bile as was
most strongly surmised.

Sep 16 2023

New refractometer is in - TSP03

PPT = 10
SG = 1.053

A very useful meter
PPT range is 0-150
SG range is 1.0-1.114

It obviously a high level of solutes are in the TSP.

---

* I need to see if the protein color reagent VIS method will detect the alteration of TSP by enzymes. This will be valuable for later use. You also need to see how NIR changes.

Let's talk about the protein isolation & purification steps a bit more as there are quite a few involved. The general steps and methods include

1. Cultivery
2. Decanting
3. Filtering
4. pH control
5. Separation by precipitation - pH
6. Deploying pI for max insolubility
7. Evaporation
8. Centrifugation

Primary Culture Stages:

Let's step through these to some extent. A few drops of human blood are the starter, a culture product itself.

1. Culturing primarily involves the use of sugar, fertilizer ("SPONCH"), and trace minerals in Himalayan salt. Starch is an option that produces an interesting and potentially important variation, but one must be aware to take the constituent starch cells that will now remain in place, primarily in the "settled protein".

A mature culture take place in ~72 hrs @ 75-95°F, 85°F seems favorable @ this point.

A successful culture will have a foam layer on the surface and the culture will produce a fairly strong odor which speaks of acidic fermentation. The color of the culture will be light yellow-green (Fe^{2+}). Proportions are roughly 1700 ml H_2O, 2 tbsp sucrose, 2 tbsp SPONCH fertilizer (Fe^{2+}) & a trace mineral source (~1/8 tsp) of Himalaya salt. As mentioned, the addition of starch is a variable cassette. Course to employ!

2. The soluble protein strongly dominates the final constitution, probably on the order of 98% +. But potential importance remains w/ the three additional proteins formed @ the ~2% level combined.

3. Steps in isolation and purification of the TSP (totally soluble protein) - could change them to WSP - water soluble protein includes:

1. Decanting the mass of solution that dominates the cultive product.

2. Filtering that solution

This heat will turn the solution a stronger yellow.

2B. Concentrate it by a factor of ~4 w/ steady heat of ~135°F. Filter again.

3. Increasing the pH of the filtered solution from the native pH ~5.6-5.8 to pH ~6.8. This will precipitate out the vast majority of the "precipitated protein". Let the solution settle for several hours and the precipitated protein will settle on the bottom.

4. Decant the soluble protein that remains and filter it.

5. Bring that filtered solution up to the estimated isolectric point of pH ~8.2. This will create maximum insolubility of the the "WSP." Additional minor precipitation will occur.

6. Filter the solution again. It will be relatively clear @ this point and suitable for storage, further tests, incubation studies, enzyme studies, etc. It will be of amber color.

—

For the precipitated protein.

1. Decant and remove as much of the WSP as possible.
2. Evaporate and concentrate w/ mild heat applied to accelerate the process. It will be of light gray color.

—

For the settled protein

1. This is comprised of the residual of the primary culture after WSP decanting take place. It will be collected with a basting syringe since it will have "SPONCH" fertilizer coarse granule mixed in. Separation will be w/ a screen mesh

Evaporate & concentrate to desired consistency and store for further analysis. It will be a very dark gray color.

Filament colonies will begin on an approximate 5-7 day old culture on the top foam layer that forms. They are minimal in size but will suffice for initial analysis. Fully developed culture will be developed more fully @ a late point, e.g., w/ the use of agar methods.

Vit B testing on the protein & also coming up.

My Windows 8 screen is 1366 x 768 pixels
Screen size ~ 13.5" $\frac{1366}{13.5''}$ $\frac{x}{1''}$
$\bar{x} \cong 100$ pixels/inch

Ok here

A blood cell on this computer screen @ ~ 8000 x measures ~ 2.2 cm

$$\frac{\text{Captured Pixel Width}}{\text{Screen Pixel Width}} \left(\frac{2.2E-2\,m}{\text{Magnification}} \right) (1E6) = \text{Size in Microns}$$

$$\text{So measured size on screen in meters} \cong \frac{\text{Actual Size in Microns}}{1E6} \cdot \text{Magnification} \cdot \frac{\text{Pixel Width Captured Image}}{\text{Screen Pixel Width}}$$

So a blood cell : $\frac{(8)(8000)}{1E6} = 2.75$

And to get actual micron size furthermore we mult by

$$2.75\left(\frac{3584 \text{ (pixel width of captured image)}}{1366 \text{ (pixels of screen shot)}}\right)$$

$= 2.75(2.62) = 7.2\ \mu m.$

There are two ways to determine the screen resolution of the computer:

1. Control Panel - Display Properties - Resolution

or

2. Capture the screen and did, open in Photo Viewer, and note pixel size.

They both match on the screen.

My newer computer has a resolution of 1920 x 1080 Hz. A blood cell on the screen @ 100% measures 3 cm

$\frac{3E-2}{8000}(1E6) = 3.75 \qquad 3.75\left(\frac{3584}{1920}\right) = \underline{\underline{7\ \mu m}}$ OK

So you must always take into account the ratio of the captured image width in pixels / width of screen in pixels

or calibrate the microscope w/ a ~~known~~ specific computer. They will both give you the same result.

Therefore, your equation is correct
i.e, relationship:

$$\text{Actual Size in Microns} = \text{Measured Size on screen in meters} \cdot 1E6 \cdot \frac{\left(\frac{\text{Screen Pixel Width}}{\text{Captured Image Pixel Width}}\right)}{\text{Magnification}}$$

$$\text{or Magnification} = 1E6 \cdot \frac{\text{Screen Pixel Width} \cdot \text{Measured Size camera}}{\text{Actual Size in Microns} \cdot \text{Captured Image Pixel width}}$$

Now if you measure in cm, (more practical)

$$\text{Magnification} = \frac{\text{Measured Size in cm} \cdot \text{Screen Pixel Width} \cdot 1E4}{\text{Actual Size in Microns} \cdot \text{Captured Image Pixel Width}}$$

eg on this computer: $\frac{2.2\text{cm}\,(1366) \cdot 1E4}{7 \cdot 3584} =$

$$\text{Magnification} = \frac{\text{Measured object in meters} \cdot \text{Captured Pixel Width} \cdot 1E6}{\text{Actual Size in Microns} \cdot \text{Screen Pixel Width}}$$

$$\text{eg} = \frac{(2.2E{-}2\ \text{meters})(3584\ \text{pixels}) \cdot 1E6}{(7.2\ \text{microns})(1366\ \text{pixels})} = 8016$$

ok

There is a general relationship for any computer or GN Calibrate the Computer and object together on a known image size.
Now on a more practical basis, if we measure the object on the screen in Cm, we have

$$\text{Magnification} = \frac{\left(\begin{matrix}\text{Measured object}\\ \text{in Cm}\end{matrix}\right)\left(\begin{matrix}\text{Captured Image}\\ \text{Pixel Width}\end{matrix}\right) \cdot 1E4}{\left(\begin{matrix}\text{Actual Size of Object}\\ \text{In Microns}\end{matrix}\right)\left(\begin{matrix}\text{Screen Pixel}\\ \text{Width}\end{matrix}\right)}$$

$$\text{Mag} = \frac{(2.2\text{cm})(3584\text{ pixels})(1E4)}{(7.2\text{ microns})(1366\text{ pixels})} = \underline{\underline{8016\text{X}}}$$

OK

$$\text{Actual Size of Object in Microns} = \frac{\left(\begin{matrix}\text{Meas. Object}\\ \text{in Cm}\end{matrix}\right)\left(\begin{matrix}\text{Captured Image}\\ \text{Pixel Width}\end{matrix}\right) \cdot 1E4}{\left(\text{Magnification}\right)\left(\begin{matrix}\text{Screen Pixel}\\ \text{Width}\end{matrix}\right)}$$

$$\text{eg } \frac{(1E4)(2.2\text{cm})(3584\text{ pixels})}{(8000)(1366)} = 7.2\text{ microns}$$

OK

Reviewing paper of ~~by~~ : Len Berz

Ok, he is using the objective magnification alone.

Ok, his red blood cell full screen measures ~ 3 cm so in the end his magnification is alone ~ 8000x

His "particle" measures ~ 0.2 cm

His ratio of Captured Pixel Width / Screen Pixel Width on his computer is essentially identical to mine eg $\frac{3584}{1920}$.

So the size of his "particles" is

$$\sim \frac{0.2\,cm\,(3584)(1E4)}{8000\,(1920)} = 0.45\,\mu m$$

Match

If the TSP is too concentrated, it will distort the spectrum

TSP02 will use only ~ 100 ul in 3ml cuvette.
TSP02 is too concentrated for both the UV spectrometer and the refractometer

TSP02 w/ a very slight amount of L enzyme added only require ~ 50 ul for the UV spectrum

—

Lets delete TSP01 and acquire a measurement for PPT & SG.

Beaker 2.70 gms + 2.0 ml H_2O = 4.70 gms
~ +2 ml TSP01 = 6.70 ml
50/50 mix

Interesting observation: When you add H_2O to TSP01 drop by drop, you can see a reaction (short term) from the TSP01.

—

Refractometer still too dry @ 50/50. Delete again.

Added 4 more ml H_2O. ≅ 10.60 gms total
so 6 ml H_2O, 2 ml TSP01 3 to 1 ratio.
GOT IT.

PPT = 68 with a 3:1 dilution ratio.
SG = 1.051

The mean TSP01 is ~ 3 times more concentrated than TSP 03 is.
It is helpful now to have an objective measure of concentration.

—

Ok, now let's use VIS spectrometry w/ the protein reagent and see if we can detect an enzyme influence,

Valley λ Samples:
1. H_2O Control
431.8 nm 2. Reagent - Reference
433.9 3. TSP03 - Concentration = 1 Use 300 ul
Adjust in λ as noted.
440.6 4. TSP03 - 300 ul + ~~300~~ 200 ul
TSP + L. Enzyme

Well the good news is that even a further shift & the right is observed.
Absorbance curves are all @ ~ same magnitude
very good.

Ok this is extremely valuable if we can verify this procedure and effect. It means that you should be able to determine enzyme impact upon the TSP. Even w/ VIS spectrometry — very helpful.

—

Now I am going to run a difference VIS spectrum between TSP 03 and TSP 03 + Enzyme from 400 to 980 nm.
Difference the time will be TSP 03, not H_2O.

—

We definitely have some differences showing up in the spectrum. You will need need a control on the enzyme + reagent alone. However, we have:

968 peak alkyl alcohol (962)

Not useful or unique — 914 Valley (lowest absorbance) Methyl (915)

Not useful or unique — 875 peak ArCH Aromatic (876)

550 peak
516 secondary peak
471 Valley (low absorbance)
} Use this region in VIS spectroscopy.

Not useful or unique — 407 peak

Now potentially the shown guide a lot of impact for the enzyme.

The shown potential difference in
1. Alkyl alcohol creation.
2. Methyl group destruction
3. Ar-H Aromatic C-H creation

by the enzyme. The could obviously be very important, the could be in addition to Tyrosine disruption.

Let's save the plot and then look at the enzyme by itself in the VIS protein report.

There are weak signals but they also are very detectable.

There are very many Cant difference that remain when the control VIS plot of the enzyme alone is Considered.

Both UV & VIS are important & conclusive.
VIS spectroscopy is useful for enzyme impact.
Here are the difference that the TSP + enzyme (diff plot), has made from the enzyme control VIS plot:

1. The enzyme valley @ ~441 nm has been shifted to the right to 471 nm. Significant.

2. The TSP + enzyme diff plot has a very modest peak @ ~516 nm. The enzyme alone control has a very strong high amplitude peak @ 518 nm. A major magnitude difference. Significant.

3. The control enzyme has a secondary also very strong peak @ ~543 nm. The diff L enzyme + TSP has a quite modest peak @ 553 nm.

Any other segments of the VIS spectrum ~~480~~ 400 to 980 have no significant difference.

Conclusion: The TSP + Enzyme diff plot from TSP
* Control alone plot shows significant impact
* to TSP 03 from the L enzyme, and the
* existence of this change is determinate by VIS spectroscopy in the range of 400-650 nm
Diff plot in this region is quite real

1. Need MR plots of TSP vs TSP + Enzyme.

2. Need to know if enzyme affects culture process.

3. Synthetic biology course

Sep 18 2023

Third Paper in progress - Crystal Biology

Need NIR sample of TSP vs TSP + L enzyme
Need L enzyme (in a culture (small is adequate))
There is now a VIS method of enzyme impact detection.
Need to isolate crystals & centrifugation
NIR of isolated crystals vs TSP
Impact of TSP upon blood was of much interest.
Impact of TSP+L enzyme upon blood - any difference?
Vit B impact upon culture, blood?
Great courses available.

Recall on H_2O_2 reactions ...

Paper #3 is written
The next paper scheduled is characterizing the TSP

There is a term to regroup and pause.

Accurate magnification is a critical issue now due to study of surface layer (it now looks like blood cells) on TSP01 after 2 week incubation.

We have known blood photographed @ 8000x (presumed)
These cells measure on the Wiz 8 computer (screen width is 1655) ~6 cm @ 100% on the screen.
Our image is 3584 pixels wide.
~~By our previous work:~~
By direct ~~estimation~~

~~Mag =~~ ~~7E2~~ $\frac{6\ \cancel{4}E-2}{7E-6}$ = ~~5100~~ Mag = 8571x

OK, so this is reasonable. No screen sizes are required with this method.
We might even say $\frac{6.5E-2\,m}{7E-6\,m}$ = 9200

and this is not unreasonable.

So the direct method is, with a known object dimension
1 enlarge to 100%
2. Magnification = $\frac{\text{Measured size in cm} \cdot 1E-2}{\text{Actual size in Microns} \cdot 1E-6}$

or

x 3. Magnification = $\frac{\text{Measured size in cm}}{\text{Actual Size in Microns}} \cdot 1E4$

or

4. Actual Size in Microns = $\frac{\text{Measured size in cm} \cdot 1E4}{\text{Magnification}}$

Our objects measure 4.5 Cm.
Therefore our size estimate is on the order of

$$\frac{4.5 \cdot 1E4}{8000} = \underline{5.6 \text{ microns}}$$

It is in range. But notice most cells are elliptical. Measuring 5 x 4 cm.

$$= \frac{5 \cdot 1E4}{8000} = 6.25 \text{ microns by } 5 \text{ microns}$$

We measure 5 Cm.

$$= \frac{5 \cdot 1E4}{8000} = 6.25 \text{ um} - \text{there it is.}$$

They are red blood cells w/ CDB centered within.

Sep 19 2023

Well, TSP 01 is absolutely producing red blood cells. It is a surface layer on the solution approx 2 weeks into incubation. Thus far even looking like blood visually. Taking place after crystallization, at least thus far.

It is actually forming a full clot network complete w/ filaments and a polymer bindy material.

My blood is looking relatively decent. The cell membranes are very much intact. Free standing cells have full integrity. The CDB serves as an attracting agent to other cells - some type of chemical, electrical or magnetic charge. The posted paper shows this well.

You need to measure and log the TSP pH'S.

You probably should not make the TSP01 blood clot NIR card until you see if you can replicate the event w/ TSP03.

TSP02 is burnt soda from overcooking, & do not expect much from it.

Let's pick up the pH of TSP:

TSP01	7.3	(not same as blood)!
TSP02	6.6	
TSP03	8.2	

Now I am of a mind to think that the pH level is going to be very important for any success w/ blood clot replication. Monitor this very closely and adjust as needed. Recall that when you create the final TSP by raising pH to 8.2 for PT level, theoretically your end point is too high. But it may be correct for crystal formation.

So let's monitor pH closely from now on in the TSP incubation process.

TSP04 is now in preparation. Currently @ precip level of pH = 6.8 for precipitation purpose. After separation, we will bring up to 8.2.

TSP05 Culture has just been started. Well probably dispose of TSP02 when TSP03 is finished.

What do you think are the chances of TSP01 being @ pH 7.2 are? Perfect biological pH for existence of blood, and in this case, creation.

I think more than fortuitous...

This is a remarkable event. Full form blood lot creation in the laboratory.

---

3 Papers on Tap:

1. TSP General Characteristics
2. Enzyme impacts
3. Synthetic Blood & Clots

Sep 21 2023

Jan 2019 (Jan 12) tremendous progress was made in determining the molecular weight of acids, including tripiotic acids. This was exceptional work. Four different acids achieved.

On Jan 12 2019 you worked w/ the soluble protein.

I also completed electro conductivity titrations as an alternative

On Jan 15 2019 we have a very interesting comment. We state that we have two equivalence points @ pH ~ 6.4 & pH ~ 10.5

Well, isn't it interesting that I am seeing in essence two levels of precipitation that have caught my interest. The first is @ pH ~ 6.8 and the other is pH ~ 10.2

~6.8 precipitates out the first protein.
~ The is @ ~10.2 & see another level of precipitation take place when I determined "maximum insolubility".

The I add a MAJOR HEADLINE note
when on Jan 15 2019 + determine the
polyprotic acid to be that of phosphoric
acid.

The look to be in Vol 25
Yes, we have notes of our debate between
sulfuric and phosphoric acid
/ and actually "sulfurous acid"
which changed the picture entirely

Major intense work took place on Jan 15
2019. On the data + story conclusion
we reached that phosphoric acid is
a match. Ultimately there was no
other choice. H_3PO_4 - it is "syrupy"

The work was quite profound + complex - It
* does not need to be updated.

We can state w/ confidence that phosphoric
* acid is a component of the water soluble
protein

"Bate" software was also important here

Look @ our notes for the hazards & damages from phosphoric acid:

1. Lowers bone density
2. Triggers major kidney issues
3. Decreases Ca, Mg, Fe, Zn
4. Increases body acidity
5. Damages tissue

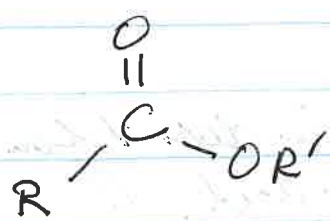
Vol 25

Major work occurred in mid June of 2019 phosphoric esters, ArCH, & plasticizers. Similarity of phosphorus & nitrogen in the periodic table.

You obviously need to look up on this ester-plasticizer - PVC issue.

Then I transitioned into EDTA titration

Ester is



They are derived from Carboxylic acids

Inorganic acids such as nitric acid, sulfuric acid and phosphoric acid form esters. Esters of phosphoric acid are especially important.

OK, get this. Esters of phosphorus acid
from the backbone of DNA molecules.

Phosphate esters are also known as "organophosphates".

Many genetic engineering procedures involve
the splitting of the phosphate diester complex
(NOV 08 2015 – Rarmwater Analysis"

Aromatic phosphate esters are plasticizers.
Aromatic phosphate esters react w/ metals,
especially iron.
Aromatic phosphate esters are typically
used in PVC
"–polyvinyl" ?? NIR

–

We have several lab notebook entries
on esters.

A phosphate ester is derived from an alcohol
and phosphorus acid.

OK, we see that TSP03 + Isopropyl ~~alcholo~~ alcohol form a white precipitate upon centrifugation.
It is completely uniform under the microscope and may well be a polymer.

There is a lot going on in the lab. The topic underway now is to size up the nature of the TSP. There seem to be a lot of features.

1. Acidic – Phosphoric acid
2. Forms synthetic crystals
3. Forms blood and clots (definitely a polymer behavior
4. Appears to form (also filaments) and appearance)
   a polymer upon combination w/ alcohol
5. Tyrosine appears to be present
6. pH estimated @ 10.2 bases side chain
   such as histidine are implicated
   Blood uses histidine for oxygen
7. Electrical conductivity is high
8. TDS, ppt is high Specific gravity ~1.05
9. Texture is that of a polymer upon evaporation
10. NIR plot tells us what
11. By color alone, iron is present Fe+2, +3
    depending upon oxidation level
12. Precipitate as additional protein @ pH ~6.8

13. TSP effect upon blood w/ direct contact
- microscope session

14. Highly toxic

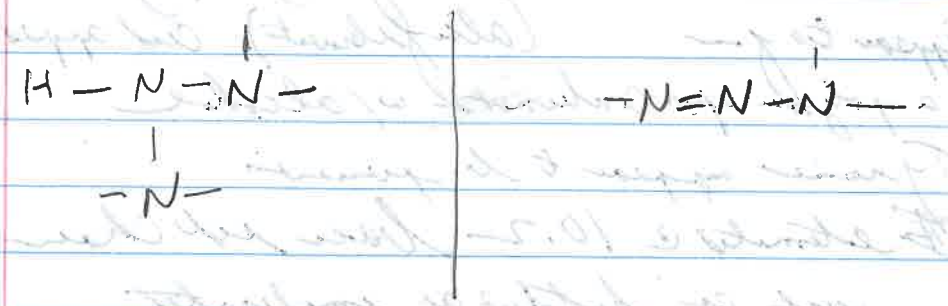
15. Inorganic analysis (preliminary, needs to be repeated)

HN_3

16. Candidates: 1. H_2O_2 ~~Hydrolysis~~ Reduction
2. Aromatic Reduction
3. Sulfite ~~and~~ to Sulfur Oxidation (Elemental)

17. H_2O_2 reaction observed:

HN_3 : ?



Need to model these and look up names

18. Phosphate Ester issue

19. Polymer candidate w/ TSP + alcohol

Sep 21 2023 (cont)

I am going to collect an NIR now. This NIR will be from the mixture of TSPO3 + isopropyl alcohol. This forms a white precipitate which can be centrifuged. The emphasis here is upon that white precipitate that has hopefully been collected on the NIR card.

The interest here concerns the relationship between phosphoric ~~ester~~ acid, phosphoric esters and the "plastic" issue. A question is whether the white precip has any vinyl nature to it. Phosphoric acid comes from Jun 2019 intense work. Phosphoric esters come from MID IR work - I believe esters came in quite strong there. Apparently phosphoric esters originate between the combination of phosphoric acid and alcohol. Let's see what we have.

I can already see that the NIR spectrum is quite different from the general nature of NIR plots that have preceded it. Let's see what we have.

Recall a ester is $R-\overset{\overset{O}{\|}}{C}-OR'$

Can we pick up a carbonyl with NIR?
We will keep an eye for that.
Let's look @ both peaks and valleys from now on and it will give us more info to work with. (1) weak (3) strong

PEAK (IS) 931nm(3) Methylene (3) (930) (930) ~~PEAK~~
No competitor, strong signal on hydrocarbon chain.

VALLEY (IS NOT) 1105 (2) No CRC candidates 2012

Peak (IS) 1186 (2) Methyl (1194) PEAK
another indicator of hydrocarbon

Peak (IS) 1339 (1) Methyl (1360) Weak signal Peak

PEAK (IS) 1372 (2) Aromatic Hydrocarbons (1370) Peak
CH Methyl associated with $ArCH_3$

PEAK (IS) 1401 (2) CH Methyl associated with branched (1396)
aliphatic $RC(CH_3)_3$ or $RCH(CH_3)_2$
Methylene (1395)

VALLEY (IS NOT) 1435 (3) NH primary aromatic amine (1432) VALLEY IS NOT

PEAK (15) 1529 (2) C-H alkyne R-C-C≡H (1530) PEAK

NH amide, NH or NH_2 (1530)

Amide/Protein RNH_2

NH for secondary amine (R-NH-R) (1530)

PEAK (15) 1645 (3) VERY STRONG PEAK STRONG PEAK

CH from Vinyl Group $CH_2=CH-$ (1637)

VINYL GROUPS are strong and repeat in multiple instances from 1620 - 1637

We have very strong abundance in the interval region.

The nitro methyl @ 1054 is on a sharp drop off in abundance - there much less likely.

We therefore have notable signals of

* 1. Vinyl groups (very strong case)

* 2. Hydrocarbon chains (strong)

* 3. Aromatic hydrocarbons Ar-CH_3 (actually a very notable peak) therefore strong

* The peak @ 1529 needs to be studied - amine, amide affluence

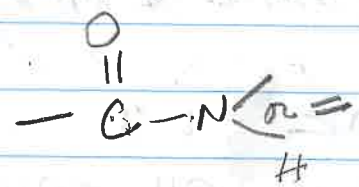
POST MIXING WITH ALCOHOL

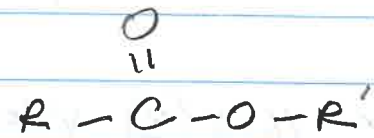
NOT MIXED W/ ALCOHOL

An amide is

Ester

$-C(=O)N=$





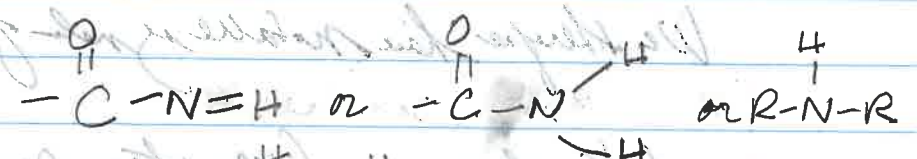
The NIR work is saying we have a plasticized amide ie,

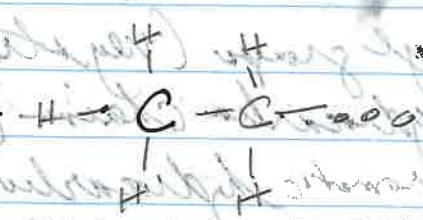
1. VINYL
2. AMIDE
3. HYDROCARBONS
   Methyl & Methylene

Combination

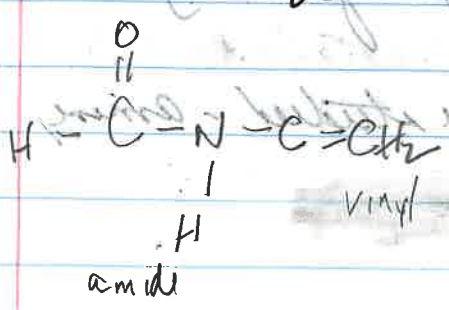
Our plot suggest

1. Vinyl $CH_2=CH-$

2. NH Amide 

3. Hydrocarbon 

4. Possible Aromatic Hydrocarbon $AnCH_3$



"Vinyl form amide"

poly vinyl amide is a target

aromatic? hydrocarbons

You should be able to construct a model of this along w/ a search for similar compounds.

Same in that of plasticized polymer amide (vinyl)

Go to work on the modeling.

This is exactly what was needed to be accomplished. We now have 3 derivatives for the TSP:

1. Synthetic crystal biology
2. ~~Arti~~ Synthetic blood - charge attractive behavior
3. "Plasticized" Polymer amide in conjunction w/ alcohol (readily available via solubility characteristics & tyrosine detection)

Model prospect

Cross Domain Bacteria: The Fallout Emerges Capable of Many Things.

1. 3 derivatives
2. Additional characteristics
3. Chemistry chain from blood - skin - coagulation

The method of determining the MW of a liquid is very interesting. You must have a bath boiling at a temperature higher than that of the sample. The solution should not need to boil, it only has to reach a temp higher than the B.P. of the sample.

Therefore, determining the BP of the sample is a very important prerequisite. I should be able to do this in relative ease.

Sep 22 2023

As usual, many important things are going on.
First focus is on:

1. Monday and regulating the pH of the various TSP's.
2. Work on boiling point determination of the TSP.
3. Molecular weight determination of the TSP is a very tantalizing prospect. Lack of equipment and proper safe lab facility both is another matter.
4. The paper is developing:
   Cross Domain Bacteria: The Fallout Emerges
   1. The three derivatives
   2. Supplemental: Physical Characteristics
      Preliminary model proposal
5. NIR Card of the crystal
6. NIR Card of the blood - synthetic
7. I have found the superb Thinkwell Chemistry/Biology courses on Udemy.

---

pH: TSP 01: Might be becoming more alkaline.
Original pH was recalled @ 7.2 on 09/20
We have 7.4 and on 09/22 we have 7.6
I need to prepare the NIR synthetic blood
Card in Case there is any difficulty or replication

The pH of human blood is very close to 7.4
I put in ~30 ml of H_2SO_4 (conc.) and it has
dropped the pH to 7.1. Stirring is active.

Let's put in 10 ml of NaOH + KOH. 10 ml additional.
Range of blood is 7.35 - 7.45. I now have
to 7.34. - Call OK.

TSP 03 is @ 8.10. Set @ 7.38

Now TSP 04 shows some activity on both bottom
and top layer. Let's inject together directly
to adjust pH.

@ this point, the surface layer activity is
that of early crystal formation.

The bottom layer is the same, but w/
protein and CO_3 development as well.
Now adjust pH. Currently @ 7.52
Set to ~~7.37~~ 7.38

So for now, the blood prospect is all
about inculcating TSP 03 & 04 @
biological pH. Anticipate 2 weeks.

Next topics are:

1. Preparation of Concentrated Crystal TSP layer

2. TSP Boiling Point investigation.
Cooking oil?

At the elevation (~4000') the boiling point of TSP03 is 99.2°C. Now let's find out what H_2O is. I will use distilled.

I need to watch a minimum fume in the lab. The boiling point is the maximum temperature reached so you do not need to be concerned when the bubble first appear.

Max H_2O temp reached is 98.4
Therefore estimated BP of TSP03 is:

$$\frac{100}{X} = \frac{98.4}{90} \qquad \frac{100}{98.4} = \frac{X}{99.2} \qquad X = 100.81°C$$

Ok, only a little but higher than water.
Cooking oil will easily handle this.

However, I suspect that the greater the Δ from H_2O, the greater the accuracy of determination. A delta of 0.81°C may not be usable.

Need to look @ the equation for error.

There is a YT version of the Dumas method that uses a syringe vs a flask to solve the pot problem. This is perfect - I think this will be doable.

$PV = nRT$

$R = 8.314 \frac{Pa \cdot m^3}{mol \cdot K}$ or $R = .08206 \frac{L \cdot atm}{mol \cdot K}$

T is absolute temp P = pressure V is volume

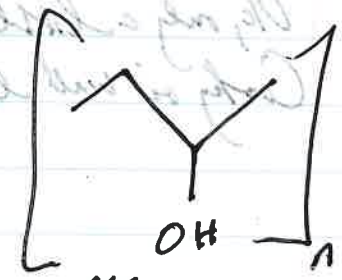
n is the no. of moles of the gas.

mass = no. of moles * molecular weight.

↑

this is what we solve for.

Polyvinyl alcohol $[CH_2CH(OH)]_n$



MP = 180-190°C

MW = 26.3-30.0

Sep 23, 2023

I have now very interesting NIR candidate between synthetic and human blood. Also between the crystal layer and synthetic blood. What is no longer available as a suitable reference for human blood.

Does human blood have a high concentration of methyl groups? There seem to be no literature to this extent.

—

I did find some Mid IR data – it is not detailed but CH_2 & CH_3 do exist in blood, apparently w/ in the fatty acids of blood serum.

—

OK, we now have a very interesting plot of human blood vs synthetic blood. Normalized and trend removal is the most meaningful. Definitely differences.

Let's look @ differences in three zones:

900 – 1100 nm

1100 – 1400 nm

1400 – 1700 nm.

We are especially interested in differences here.
First zone is ~900-1100nm.

H.B. Human blood has a strong dominant peak @ ~992
S.B. Synthetic Blood has the slightest trace of a peak there but S.B. has a sharper peak @ 915nm.

992 H.B Now, Phenolic OH (says in CCl) (990)
Primary alcohols $-CH_2-OH$ (996)

915 S.B. Methyl CH_3 (915)

So our first indication of disturbance appears to be the hydrocarbon and any OH aspects of the blood.

Our next zone is 1100-1400nm
Here we have some absorbance in S.B. @ ~1198 that is not expressed in H.B.
Also H.B shows very low (absence) of absorbance @ ~1228 relative to S.B.

S.B.
1198 Methyl (CH_3) (1194, 1195)
1228 CH or .CH Secondary or Tertiary Carbons (1225)

We therefore once again have a strong signal of disturbance in hydrocarbons and the methyl group. Synthetic blood is in 3 ways showing absorbance (as H.B is showing the lack of) of hydrocarbons and methyl groups that differ from H.B.

We also apparently have a disturbance w/ the OH group, quite likely in combination w/ ~~hydrobar~~ hydrocarbons.

Now the zone 1400 - 1700 nm

H.B shows absorbance @ 1414 nm that does not appear in S.B.
SB shows major absorbance @ 1473 nm that does not appear in H.B.

@ ~~1526~~ 1523 H.B shows minor increased absorbance while S.B shows a slight decrease.

@ ~1578 Synthetic Blood shows absorbance that H.B. does not

and

@ ~1632 H.B shows slight absorbance that S.B. does not

H.B 1414 Methylene (1411) (1415) → Possible
But now consider 992 + 1578! → Alkyl Alcohol (1416) → Not expected
Aromatic CH (1417) → Possible

yes! 10% H.B Blood is not anticipated to have alcohol ~~aromatic~~ in it so CH_2 is the likely. Phenylanine also OK.

Aromatic CH in blood? Phenylanine? Tyrosine?

Conclusion here. Reference info. says hydrocarbons do exist in blood from fatty acids.

Yes, fatty acids (lipids) are expected to be in the blood. This says that the S.B. does not appear to have such lipids, or fatty acids.

SB 1473 Major absorbance here
N-H Amide w/ NR group
* Amide/Protein (1471)
* NH Primary Aromatic Amine (1472)
*

This says that we have a major amide/protein contribution from S.B. that does not appear at the same level in H.B.

H.B. 1523 NH Amide, NH Amide/Protein (1520)
NH secondary amine (1520)

"Engineering tyrosine residues into hemoglobin enhances heme reduction, decreases oxidative stress & increases vascular retention of a "hemoglobin based blood substitute".

so tyrosine is not expected to be in the blood.

Hemoglobin positively contains phenylanine, ~ 10% by weight.

This therefore indicates our second form of likely protein disturbances in the blood.

S.B. 1578 Alcohol R-C-O-H (1580)
Alcohols & Water O-H (1583)

This says we have an alcohol in the S.B.
We know that this is forming a polymer.
This supports the finding @ 992 nm.
There are now three indications of OH disturbance possible: 992 nm, 1414, & 1578 nm

Thus far, hydrocarbon, OH, and protein disturbance,
(esp methyl) (alcohol) (2 places)

B 1632 Vinyl, Vinylidene (1630, 1631, 1635)
(also 1621, 1637)

And we now have a definite signal w/ vinyl.

So our disturbances in the blood are likely expected to be:

* 1. hydrocarbon (fatty acids), methyl group

* 2. OH group (affects solubility) amino acids.

* 3. Protein disruption, alteration

4. Vinyl or Vinylidene presence (plastic, polymer formation)

It sure fits the observations of blood clot formation & circulatory disorders.

The culture is what has allowed this to be learned. Quite important.

I now have an additional plot of great interest. A major skin eruption has developed on the ankle. There is no response to ultrasound and a balm use - the majority likely ultrasound. The skin effect is quite deep and painful, extending into the nerves.

I have a strong signal difference plot. Reference adjacent normal skin and the target is the severely erupted skin. The texture and turgor pressure of the affected skin is quite different.

1. Severely inflammed deep red, pain, nerves and itch, sometimes difficult to put weight on the foot.
2. Skin is not of normal flexibility, more like a dense resilient plantar material, like very dense memory foam.

We have a series of strong peaks and valleys. Let's examine:

(1) weak 3 (Strong)

Peak (IS) 966 (3) alkyl alcohol (962)
OH from non hydrogen bonded
short chain alkyl alcohols

No other competitor here.

The likely effect on interpretation of the 1414 peak in the previous Lethal blood vs synthetic blood comparison.
(We know now that the TSP reacts w/ alcohol & produce a polymer)

Valley (IS NOT) 1109 (2) No CRC 2012 listing

Double Peak (IS) 1164, 1186 (2) Double or Broad Peak
C=O Carbonyl - Aliphatic Hydrocarbons (1160)
No Competition
Polyenes, alkenes (1170)
CH_3 Methyl Aliphatic hydrocarbons (1194, 1195)

Valley (IS NOT) 1277 No CRC Listing 2012

PEAK (IS) 1384 (3) Very strong peak here.
Methyl (1370)] aliphatic hydrocarbons
Methyl (1390)]

Valley (IS NOT) 1453(3) Very deep valley here - lack of absorbance in affected skin

Aromatic Amine (1452.5)

WATER 1453

We have seen this before. Water is severely removed from the skin and removes elasticity.

Broad Peak 1590(3) Peak is broad here (1560-1590)

Polyamides (1570)

Amide / Protein (1570)

This indicates the presence of a significant foreign protein in the affected skin.

Peak (IS) 1622(2) Vinyl (1624)

$CH_2=CH-N=C=O-C$

Vinyl on pyrolidnone

CH Acrylate (1621)

Alkenes (1620)

Polyamide (1618)

Peak (IS) 1676(1) Methyl (1676)

So what by nature do we see here in the affected skin?

Likely candidates are:

1. Alkyl Alcohols — OH
2. Methyl — Aliphatic Hydro carbons
3. Severe removal of water, elasticity of skin
4. Foreign protein in the skin.
5. Vinyl

Exactly the same disturbances are seen when Comparing the synthetic blood to human blood.

What is happening in the blood is happening throughout the body and it can emerge from the skin under certain conditions or protocols but if you do not get it out of the body through the skin it will inevitably lead to increased blood coagulation or clotting.

Sept 23 2023

A fourth form has now appeared in the TSP isolations. TSP.04 is developing a surface layer w/in 48 hrs that has a quite large organic but unusual life form. 3200x - quite large.
It has a buddy structure to it however it contains w/in all of the complexity ever seen w/in the filament structure.

TSP is hardly just a "protein"; it is a complex that allows for multiple expression of
1. Crystal Biology
2. Artificial blood & clotting structure
3. Polymer formation in combination w/ alcohol
4. A relatively large organic life form

Also we know that functional groups involved are
1. Hydrocarbon - combustion test is consistent
2. Alkyl alcohols
3. Proteins
4. Polymers such as vinyl.

Sep 24 2023

Plans include Torch, spoon

1. Flame test on dried TSP - hydrocarbon content
2. Test for alcohol?

3. Molecular weight determination of TSP possible?
4. The paper started?
5. Cation test kit? Anion

6. TSP inspections - pH monitory
7. Anesthesia project

The TSP incubations do not necessarily maintain a steady state or exactly repeat themselves.

1. TSP01 seems to have reached a steady state, however. I see no additional activity taking place. There remains one small residual portion of the synthetic blood development. The growth has not extended nor appeared in the other TSP's 03 & 04. TSP01 appears to have reached an end point after ~ 2½ weeks

2. TSP02 is for spall studies, such as drying and a planned flame test.

3. TSP03 seems to be the most inactive of the point. BUT
w/ one very important section. A dense crystal-filament mat up to 1 cm diam has formed on the surface. It had adhered to the lid of the vessel during movement and was not initially visible in the liquid surface. It has been photographed. It is quite dense. In addition 3-4 isolated blood cells were found, flat and difficult to find. Photographed @ 800x. This does represent the 2nd TSP inoculation that has now been observed to present red blood cells so there is synergy, can't that it has repeated, even though mineral is present in TSP03.

4. TSP04 is also very intriguing. The surface layer of relatively large bladdery structures, depicting a new variant-type form, that appeared last night has now disappeared.

The main variation that I am aware of with environmental conditions is that of temperature. The night are fairly cool and the trailer is cooling down to ~ 50°F, ~ 35° outside. So this may be affecting the cultures.

I would like to set up a run of TSP04 held @ ~ 80°F and pH 7.4 if possible - I do not have these conditions yet but will by in the warmer.

80°-98° is in range

TSP 04 bottom layer is now compared of (~ 48 hrs)

1. CDB in large number
2. CDB in Chain form (greater number and longer chain than prior)
3. Protein mass
4. Ammonium crystals
5. No blood cells or developed filaments visible

We now have a minimum of 8 9 known variants that emerge from TSP inoculation

1. Immature crystals of irregular geometry
2. Mature "exotic" crystals
3. Polymer webs mixed w/ alcohol
4. Red blood cells, polymer-filament matrix
   - essentially an arterial clot
5. Large muddy organic forms
6. Chain CDB formation
7. Protein mass - heavy iron content likely
8. CDB
9. Clear filament formation

Condensed TSP is indeed combustible. With slight fluid remaining, it burns similar to tar.

Also, combustion of the TSP (small amount) produces significant VOC's. On the order of 6 mg/m3 (500+ PM 10). Per research with pyrolyze indicates significant toxic effects from combustion.

VOC's are also detected in the drying process, ~ 0.1 mg/m3

VOC's:

0 - 400 ppb Considered "safe"

Short term effects headache, nausea, dizziness, irritation of respiratory tract + eyes @ 400 - 2200 ppb

6 mg / m³ in ppb.

mass of air in ~ 1.204 kg/m³

= 1204 ~~mg~~ mg/m³ ~~1204~~

= ~~1204E3~~

= 1204 E3 mg/m3 $\frac{1204E3 \text{ mg}}{6 \text{ mg}} =$

= 2.01 E5

so 1 part in 2.01 E5

in ~~ppb~~ pp thousand = 1000 in 2.01E8

in ppm = 1E6 in 2.01E

in ppb = 1E9 in 2.01E14

So $\frac{1E9 \text{ ~~ppb~~ parts}}{2.01E14 \text{ parts}} = \frac{X}{1E9}$ $X = 4$ =

And we need 1/400. So roughly ten times over safe level.

Short term effects @ ~ 1000 ppb.

We are ~ 5 times over short term adverse effects. Obviously harmful.

if we want to have 0.1 mg/m3

$\frac{.1}{6} \cong .017$ & $.017(4975) = 83$ ppb.

Considered to be ok, but I certainly would not trust it over time. You need to keep VOC's < 0.1 p. mg/m3 and keep it short term @ that. You can be sure that VOC exposure, however small, over longer duration is hazardous.

So burning of that small amount of TSP exceeded safe exposure limits by a factor of 10 - 20 times. Bad news.

It appears that evaporation of the TSP @ 135°F creates VOC's on the level of 0.5 mg/m3.

Conversion 6mg/m3 ≅ 4975 ppb ≅ 5000 ≅ 6000
Therefore multiply mg/m3 by 1000 to get ~ ppb.
eg 0.05 mg/m3 ≅ 50 ppb
Considered reasonably safe on the scale of 0 - 400 ppb
0 is best of course if you can get it.

I have relocated the qualitative tests for ions by "Doc Brown" - these were always my favorite.

Need BaCl or $BaNO_3$

very expensive Silver nitrate for Cl⁻ ion

Nitric acid

Litmus paper

Fine aluminum powder - Nitrate ion

Nitrate, Nitrite - Aquarium kits

Let's think about the cultures. Recall that TSPO1 was the first generation. As such, it likely did not have any starch added. I think we may need to back up to that @ some point.

Molecular mass 'lab. Example'

Eg 0.3 gms of liquid.

Sep 25 2023

I am conducting a rapid retracement of the culturing process. Variables involved are

1. Starch or no starch
2. Precip or no precip
3. pI - pH treatment or not.

The reasoning is then. TSP01 is the only culture that demonstrates clearly the more advanced growth of exotic crystals as well as synthetic blood visible.

TSP01 was the early generation culture so we must consider the above as a strokey luck. Now recall that TSP01 had a pH of 7.2 when the synthetic blood was discovered. This implies that the precip adjustment of that culture must have been made since the native pH is ~ 5.6.

* But it does not say that starch or the pI investigation was applied to it.

* Therefore these steps need to be retraced. We also have the physically that ~ 2 weeks of incubation of TSP01 was required for synthetic blood appearance, & under warmer temperature @ that.

Therefore we must quickly retrace.
Sample production will be of smaller volume
w/ greater variation on the factors.

I now have a TSP 4.5 on hand.
TSP 4.5 has starch in the culture but
no additional treatment as of yet.

Upcoming: TSP 05 will have no starch.
TSP 06 will have starch.
(~3 day development period)

VOC's are now observed in correlation w/
culture production, fortunately @ low levels.
Combustion is, of course, an entirely different
matter but that is not planned beyond
the single trial required.

Guess what? TSP 01 continues to have
significant synthetic blood formation.

* We must reproduce TSP 01 conditions

TSP03 has a large mass crystal surface layer which has been photographed and it does offer a contribution.

TSP04, however, seems to be the least productive of all. Although incubation time is short, I expect to see some great activity.

—

Let's work to recreate TSP01. The primary unknown may only be the starch source.

—

Recall that I have adjusted the pH of all incubations of TSPs to biological pH of ~7.4.

Now, as expected, TSP4.5 has a pH of 5.8. We know, therefore, that protein precipitation was conducted to bring the pH to 6.8. Let's try to review any notes I have.

—

I took rather decent notes, and these will likely be helpful. Culturing began on Aug 19, 2023. (Vol 30) The work is going to lead to the designation and identification of TSP01. Let's see what we did.

I have very good & useful notes.
I have the TSP showing up in a 72 hr culture (no starch). pH had reached 5-6
Great. I am just beginning to realize that I have the TSP coming into existence

—

On Aug 21 I already began investigating the starch role (potato). I also began to investigate filtration. & also input a very productive culture in place w/ the introducing the potato.

Now, shortly afterward I begin to dismiss the potato introduction as a "distraction" to the culture process.

It appears that I return to the original mineralized culture w/ no potato involved.

—

I then know that I can precipitate a protein out of the culture liquid by filtration. Potato is declared removed on Aug 23 from the process.

I believe our focus now falls upon the precipitated protein vs the remaining soluble solution. No "TSP" nomenclature has been introduced yet.

On Aug 25 I begin comparing the remaining "solution" (TSP) w/ the precipitate. I realize that I also have protein within the remaining solution after precipitation is separated.

Next I start looking @ enzyme compact. I determine tyrosine w/ the "soluble protein" Aug 27

Aug 28 - I am clearly working with the "soluble protein". Titration active under this condition. Solid & soluble protein forms clearly noted. I now take the pH up to 10.5 Potato role is again questioned.

Aug 28 - I use the term "TSP" for the 1st time.

Sidenote: Within 2 hrs of culture start we now have VOC's appearing. 0.03 mg/m³ ≅ 30 ppb.

What I then do is centrifuge the filtered solution from culture. No starch is beg used to the best of my knowledge. Centrifugation separates out a layer which by microscopy we see identifies as PURE COB.

The remaining solution is what for the 1st time we designate as TSP. We then say that precipitation and pK(pI) analysis
* will be applied to that remaining decanted solution.

Aug 28 (Cont) NIR plot between separated COB and remaining solution is of interest because the NIR difference is insignificant. A confusing finding. Implication is that centrifugation is not critical.

(I know that centrifugation has not been applied since that time)

Aug 30 Precipitation claimed to be successful and productive & centrifugation not required.

Note: we clearly implicating that no starch is being used & ppt precipitation from pH ~5.1 to 6.5 is a critical step of separation.

I actually mention VOC production on day 31 at the same level, ~ .05 mg/m³.

We have the TSP product pH ~ 7.0

We also start referring to it as a "polymerized protein" that is water soluble, vs a "water soluble" protein. Actually more likely the case...

Step 02 - Now we start conducting pI analyses. This is important to know. We still call it "TSP", not TSP01.

Now remember, I would never use the full sample for any one test, it would be using a separated subset of the original sample. So pI will not be active on the entire sample.

We specifically mention the strategy on Step 06 Our pH of the native TSP is ~ 6.5.

Sep 06 Crystalline geometry stated @ pH 6.8
No pI applied!

On Sep 09 I refer to TSP 02 which we
now know was overheated and only
acts as "spar" material.

There TSP 01 was all in place by Sep 08
w/ Crystal biology active.

Status of affairs pH ~ 6.8
No pI titration
No starch expected - Correct

Enzymic work become active
TSP impact upon Contact w/ blood

Starch use is now mentioned in use
again on Sep 12.

Ok, on Sep 12 we have a rather startling
statement:

Sep 12 Notes:
Ok, this gets difficult to sort out. I am looking @ different cultures but writing about them as if they are all the same. Clearly not the case.

1. I talk about using starch again. I like what I see on productivity and I raise the pH to via precipitation from 5.6 to 6.8
I CALL THIS TSP03.

TSP03 thus far shows itself to possibly become very unproductive w.r.t. Crystallization and synthetic blood.

Then I start to advocate for the modified culture that includes starch.

BUT THEN I make the comment of observing erythocytes — I specifically refer to TSP01 as the culture of observation.
It looks like our range of time
Aug 28 TSP01 must be in place
Sep 08 Crystal Biology active
Sep 12 Synthetic Blood observed
~Sep 20 pH adjusted to ~7.4
Sep 25 Increased synthetic blood appearance after all visible material had been used in tests.

Therefore the culture has essentially a 1 month life span and shown itself to be completely unique.

You do not have this amount of time to try out different scenarios - it appears that it will require 2½ to 4 weeks to recreate.

Our best hypothesis is:

1. No starch, minimalist culture (we will run simultaneous tests)
2. Precipitation conducted w/ pH raise from ~~to~~ 5.6 to 6.8
3. There may be no need to siphon off the precipitated protein, the only important factor may indeed be pH.
4. Final adjustment of pH to 7.4 w/ strong base.

What this means is that we may simply be able to carefully bring up filtered solution to pH 7.4 and that is it.

There should be no reason required to separate out the precipitated protein but it might help for observation purposes.

Lets start running smaller tests now.
I have 4 suitable culture containers.
Focus on no starch but for now I have starch sample material. Start the series as TSP 10 & TSP 11

TSP 10
1. Starch
2. Direct raise of pH to 7.4
3. Incubate
4. No precipitate separation

TSP 11
1. Starch
2. Precipitate to pH 6.8
3. Decant & separate
4. Fine tune pH to 7.4

Split the sample: Total = 671 gms
Jar mass = 247 gms Δ = 424 gms / ~~2~~ 4 = ~~212~~ 106 gms each
Vessel Mass = 221 gms 221 + ~~212~~ = ~~433~~ gms each
106 = 327 gms each

No, lets change our plans. Divide into 4 samples, not 2. 300 gms each is plenty.

Ok, the well has much more production to zero in on TSP 01 conditions.

The other two TSP cultures, TSP 03 & TSP 04 do not matter very much right now. TSP 03 has some value as it has produced a surface mat layer.

TSP 04 can actually be scraped right now as shows no known significance.

TSP 01 is now turning RED throughout the entire solution, so blood production has undoubtedly become very active. pH is likely the most important measure.

*

—

I need to increase the pace of and the efficiency of synthetic blood development. I now have 5 trial cultures available to me to work with, TSP 10-14. Each one contains ~88 ml of filtered culture solution (no other processes involved, pH ~ 5.6) (212 gms vessel + 88 gms solution = 300 gms total Easy to work with - 5 trials per pint)

Now let's start setting up trials while the next batch cooks, 1 w/ starch and one w/out starch. Hopefully ready in ~ 72 hrs 65-95° F.

TSP 10 - Starch, direct increase of pH from ~5.6 to 6.8 to 7.4 w/ H_2SO_4/NaOH.
27 drops Conc. NaOH - KOH → 7.0
+50ul → ~~7.35~~ ~~0k~~ 7.8
Cola change from white to brown brown w/ ΔpH of 7.3 to 7.8 No precip separation.
* Notice this / The Cola change could be important.
Let a trial to stop @ pH 6.8 so that it does not turn brown.
Culture look to be very reactive & pH between 6.5-7.5
Reduced pH to 7.32

TSP 11 Direct increase pH
20 drops. 5.8 → 7.40 → 7.34 got lucky.
Remained yellow.

TSP 12 Direct increase pH
18 ~~15~~ 10 drops 5.9 → 6.85

TSP 13 Direct pH
5.8 → 6.80
Double Filtered

TSP 14 Direct pH
5.8 → 7.55 Turns brown!
Double filtered

—

* The lesion for today: the TSP appear to be extremely resistant to change @ biological pH.

—

Undoubtedly the filtered solutions will be much easier to inspect; BSA will hold the set for now. 3 unfiltered trials, 2 filtered trials, encompass pH range from 6.8 to 7.55 should be satisfactory.

—

Now for TSP 01 stock:

Blood has once again been confirmed. TSP 01 is showing a resurgence of blood growth since adjusting to biological pH.

The TSP solution slowly is turning red and a fairly substantial network of blood has now formed up in the solution.

The cells must be viewed to view properly. It appear that the polymer matrix obscure the clarity of viewing the cells.

Everything related to synthetic blood now rests on TSP01. I am attempting to repeat the event w/ 5 separate TSP trials that vary by slight pH changes and precip pelleting yes or nay it is indeed remarkable but only TSP01 produced the event over 2½ - 3 weeks of time.

Let's test the pH of TSP01. 7.49

Sep 26 2023

TSP Inculation Inspection

TSP 03 Trial w/ Crystal flake on surface. Surface layer shows iron protein complex and short chain CDB formation. pH 7.38

TSP 10 Unfiltered. pH 7.32
Extensive immature crystal layer forming on bottom. ~~Nothing visible on surface.~~
This level of crystal formation is therefore taking place in < 24 hrs.
This looks to be productive. Actually there is some surface material but not overly distinctive @ this point. Some immature crystal formation. pH here went too high and was then lowered.

TSP 11 pH here did not exceed the biological threshold. Much more yellow. definite surface layer. One way pH up to 7.34
TSP 11 is already looking to be an important culture. Crystals on surface are much larger and also show greater geometric complexity
Internal organization of CDB complexes are readily visible.

These indicate that it may be extremely important to not exceed the biological pH, but that it will need to be a one way increase from the native pH of ~5.8 up to biological pH of ~7.4. Our measured pH stopped @ 7.34. A great deal of external crystal organization has taken place within 24 hrs. There surface layer matured only, reading results.

Bottom Layer: immature crystals with extensive protein complexes.

The culture to be watched closely. Unaltered one way pH increase.

TSP12 pH here was kept lower to 6.85 and left unaltered. Generally a low level of development. Surface matured not as much, bottom layer appears similar to TSP11.

Recall that starch is included in each of these treats. Starch in no way is unhelpful what is taking place in TSP11.

TSP13 has a low pH 6.80, double filtered
Very low activity rate, more on surface.
Slight slight activity settled on bottom.

Very low level of sophistication and activity.
Generally non productive even though
it looks good as a very clear solution.
It is incomplete.

TSP14 High pH of 7.55, Double filtered.
Basically shows no visible activity
and is a darker color. An inactive culture.

A great deal has been learned here
from the set of five trials.

Only one culture can be considered productive
@ the level desired, and that is TSP11.
It tells us some very important things -

1. Starch did not hinder the culture, it
may have helped. We shall see.
2. No filtering past precipitation is
to be more beneficial. It needs
both parts to succeed.

3. pH Control and transition is extremely critical to the entire process. The native pH is ~ 5.8. The pH must be raised gradually and smoothly to biological pH of 7.35 - 7.45. Biological pH can not be exceeded, even to 7.55. THIS IS CRITICAL

* * *

The shows that the protein complex is designed specifically to develop and function within the biological pH of blood, and that it is highly constrained to the range. Too low may be possible, eg 7.2, but too high eg 7.55 is fatal to the process.

The knowledge is paramount to understanding how the protein complex operates. There is likely another metastasis strategy:

1. Ultrasound
2. Enzymes
3. pH Control or influence.

TSP 11 is now of high priority and attention.

I think that the next steps required are:

1. Determine optimum VOC production temperature, I suspect it will be higher than 85°, maybe closer to 110°C.

2. Determine optimum TSP incubation temperature. It would seem like it should be close to 98.6F

Let's split up TSP 11 and incubate.

Also, can we improve any of the other treatments? TSP 12 & 13 may be able to be salvaged. Bring pH carefully up to 7.4 (?) and add some of the precipitated protein to TSP 13 since it was filtered.

A very good idea here and we may be able to create 3 active cultures.

Make new solutions as well stored under established biological pH.

Ok TSP 12 is now set @ ~7.40.
In addition 1/2 of TSP will be incubated seeky 98.6. Original 1/2 well stay w room temperature (48-70°F)

Now work on TSP 13.
Add ~1/2 ml of precip protein which had been filtered out

This adjustment of pH has to be done VERY carefully, in 10-20 ul increments as it is approached along w/ adequate stabilization and stirring.

—

Ok, the VOC's are starting to come in. Lower culture is @ 130°F, upper @ 97°. Very good. Culture productivity activity can be monitored by VOC levels.

TSP 13 set to pH 7.40 w/ protein precipitate added. Take 1/2 and incubate seeky 98.6F
Other 1/2 @ room temperature 48-70°F

Very good. I have managed to salvage much of the previous thread w/ a total now of 6 potential or actual viable cultures.

1. TSP101 – fully viable w/ synthetic blood
2. TSP 10 – sophisticated COB/Crystallization operative w/in 24 hrs.

3. TSP12 raised to biological pH ~7.4
   ½ in incubator steady 98.6°F
   ½ room temperature

4. TSP13 raised to biological pH ~7.4
   ½ in incubator steady 98.6
   ½ @ room temperature

I will now discard TSP03, TSP10 & TSP14.
Not regarded as productive for now known reasons.

Proper pH, nutrient and temperature control will be required to produce productive cultures.

Balm
Ultrasound
Enzymes
pH

I have learned some things about the VOC issue and I am now more sensitive to their presence. They definitely have an impact upon health. As usual, the standards of "safe" are way too high. I can sense their impact at low as 20 ppb, not 400 ppb the upper safe limit.

First, VOCs are a direct expression of the activity level of the COB cultures. Commonly they are in the range of 50-60 ppb in the room definitely too strong for my comfort level and health.

The only answer is to keep the window open, even if the room is cold (only required during active culturing).

I can drop them to zero for almost all of the time with the window 3/4 open. I think the active heating portion (for any short term) will increase them temporarily to the measurable level. Summary: Ventilation will always be required during active culture development.

Molecular mass project.

Need

Mass @ 0.30 gms $pV = nRT$

Temp @ 100°C $n = \frac{PV}{RT}$

Volume @ 62.8 cm³ (need in m³)

Pressure @ 101 KPA (need in Pa)

$R = 8.314 \frac{J}{K \cdot mol}$ $T = 373°K$

$n = \frac{(101000\ Pa)(62.8E\text{-}6\ m^3)}{\left(8.314 \frac{J}{K \cdot mol}\right)(373°)}$

$n = .00205\ mol$

$Mass = n \cdot MW$

$MW = \frac{Mass}{n}$

mass = 0.30

$MW = \frac{0.30\ gms}{.00205\ mol} = 146.1 \frac{gms}{mole}$

Acetone 46.5°C

Our expt mass = 29.12 gms w/vt + cap

29.79 w/cap

31.02 ~~32.44~~ w/alcohol (like to much)

~~30.95~~ 30.93 .60 mL

A very interesting first run on this method, attempting to use the syringe approach. Unfortunately, the method seems flawed as the syringe immediately collapses as the tube is cooled down.

Nevertheless, our numbers are (Choose 101 kPa, 90°)

87500

$n = \frac{(101000)(60E\text{-}6\,m^3)}{(8.314)(363°)\ 358} = .002008$ ~~.0017396~~ .0017159

~~363~~ 368 ~~51.7~~ ~~53~~ 52.45 ~52

[.09] $(31.02 - 30.93) = 44.82$ vs 60 .10 actual

C_3H_8O

.002008

.0017396 .0017159

Still 13% error. Not actually too bad.

Interesting. Not totally out of line. ~~25%~~ error

So if the mass was 25% less it would come out.

Basically 0.02 gms quite easy to do. Cooling longer?

What about the syring collapsing - does this matter?

Easy to do this again.

But guess what? If the pressure is less, and it is the value would come out higher.

4000' Our pressure is actual 87.5 vs 101

If temp was ~~85~~ 95 vs 90: No, that makes it worse.

It gives you a rough idea very quickly.

Sep 27 2023

1. The alcohol MW determination is to be repeated

2. VOCs will now be used to indicate culture activity

3. TSP cultures are to be inspected TSP 11-13 30ml TSP 12, 13

4. An idea exists to extend the blood culture of TSP 01 to TSP 11 if TSP 11 inspection substantiates. Splitting TSP 11 may be the best strategy here. Always maintain that which is productive.

5. There look to be a great buffer lab in my Chemtab software. Could be very useful.

TSP 11 - The crystals on the surface are quite large w/ unusual facet geometry and internal CDB organization.

TSP 13 30ml incubation shown shows no unusual activity. CDB aggregation & iron complex on bottom settled layer. We will free up this container.

TSP12 30 ml incubated 98.6 has poorly formed small crystals on bottom settled layer.
The container can also be made available.

TSP11 is our culture of greatest interest coupled w/ TSP01 w/ blood.

TSP12 & 13 are both darker in color indicating that pH threshold was crossed. They can both be removed from consideration

The goal is now twofold.

1. See if we can transfer synth blood development from TSP01 as a starter into TSP11.
Use 30 ml container. No incubation yet.

2. Carry forward what has been learned from TSP11 and now determine the starch / no starch effect.

We have no VOCs so cultures are already done. 48 hrs

The idea of bringing a small portion of TSP01 (w/ blood) into TSP11 was highly successful (30 ml incubated overnight). We have sophisticated internal CDB crystal organization along w/ synthetic blood production.

Not sustained.

We should now be able to propagate the culture more rapidly.

Sep 28 2023

Add human blood directly to favorable TSP & Kemp + pH control ?

OK, be very careful. TSP11 + TSP01 w/ synth blood has deteriorated. There is no significant culture development nor well formed synth blood growth. The idea of mixing these cultures seems as though it may have helped for over a few hours yesterday but the progress has not been sustained. The only viable synth blood culture now remains as TSP01. To be very cautious that you do not damage TSP11. It was wise to separate the inoculation trial. Visually the culture does not look successful either.

— Small rectangular long axis crystals

Now back to TSP11 inspection.

We have some amazing photographs. Some of the surface crystals are now extraordinary large, likely up to a mm or so. But what is most amazing is that these crystals are developing "red veins" w/in them. For now, do not inoculate and water the culture closely.

Difficult to collect w/ the pipette, possibly too large.

It was accomplished w/ the needle and then rinsing the needle onto the slide.

Bottom layer has immature crystals, iron protein complex, CDB & short CDB chains

Therefore the surface layer is very unique and must more highly developed. Might indicate an affinity to oxygen/nitrogen. This is when the synth blood layer on TSP 01 formed — on the surface.

We will watch this carefully, do not disturb it.

You have large volume of culture material being established soon — starch vs no starch.

Sep 29 2023

The lab continues to look better and better.
Fire protection - extinguisher + smoke detector
Cooking pans - adequate heat for stove w/ propane
Thornwell courses are back in place
Adequate storage containers/vials - small funnel

—

You are slowly & acquiring large amount of TSP
and you will need for later. Remember TSP has
a high concentration. We also want to work towards it.
TSP II has some astounding crystals w/ vivid red
venous color develop, very strange.

—

Now our big question is, of course, what exactly
are going to be the requirements that produce
synthblood?

1. Concentration
2. Biological pH? Temperature?
3. Relationship of vanadium crystals to synthblood.
4. Incubation temperature.

Can we propagate the blood into a successive
culture? (Not yet).

4. Does subsequent concentration change the
culture, I suspect so.

Let's deny note our newer culture.

~~TSH~~ TSP 12 WSA With Starch A
TSP12 WSB With Starch B

@ this the point A & B & volumes of A & B are the same.

Now TSP11 continues to show crystallization that appears to be large. I have difficulty w/ pipette collection. I have therefore widened the tip of a custom pipette to facilitate collection. The culture material is delicate, very limited in quantity so it can not be disturbed. It sure likes the predecessor to the synth blood however in that it is forming a surface network that congregates. Let's look under scope hopefully w/ a better non-disturbing sample.

Need to look @ dental anest/anesthetic

We definitely have a polymer forming — this is a good sign — this is a starch culture.

TSP 11 is becoming quite amazing in its own right. We clearly have
"Biological ~~Circuit~~ Crystal Circuitry"
in play here.
This could be the harbinger to ~~syth~~ synth blood production, will monitor this closely.

—

The bottom layer of TSP 11 continues to quite different - it appears to be the "working factory" location ie, CDB, CDB chains, immature crystals & iron protein complex.

Therefore a big difference between what takes place on the surface and what takes place on the bottom layer.

TSP 11 is a powerhouse also.

—

Let's not disturb TSP 11 now.
Let's see if TSP WS-A develops any differently under 98.6 incubation.

—

You are also wondering if citrate is going to affect the culture.

How to make citrate from citric acid?
I would think that a base is added.

—

. 80 ppb VOC from heating up the TSP
Cultur (filtered) first solution to 120°F,

—

2.5 gms Baby Soda + 2.1 gms citric acid = 2.9 gm
Sodium citrate

Also we can make sodium citrate buffer.
1. 2.1 gms citric acid in ~~but liter~~ H_2O
a little less than 1 liter
2. Now add 1M NaOH to bring pH up to
6.0 (might experiment w/ this —
could I bring it up to 6.5? 7.0?)
3. Now add H_2O to make = 1 liter

—

Indeed you can use NaOH instead of baby
soda

—

Citric acid is a triprotic acid. Very
interesting as it should have parallel
to phosphorus acid.

$3\,NaOH + C_6H_8O_7 \rightarrow 3H_2O + Na_3C_6H_5O_7$

3 mole NaOH + 1 mole citric acid →
3 mole H_2O + 1 mole of trisodium Citrate.

You also want to try to duplicate the potato cultures —

Now take another TSP 12 WS-A and add TSP 01 agar blood for a pepsect trial.

You also have a TSP 12 WSA under incubation to see if any are crystals form more quickly than not incubated.

Acid dyes will dye only protein/animal fiber. Rit dissolved, will dye most any fiber. Rit dyes are a combination of acid, direct and disperse dyes.

"Some acid dyes can be used to stain organelles in the medical field."

Acid dyes are for polyamides & proteins!

Sep 30 2023

The quite large (up to 100 μm diameter, ~1mm in length) viscous crystal biology structures in TSP 11 are quite amazing. There is various distribution w/in the structures, and it is red,

What is both amazing and intriguing is the level of variation w/in the culture series. Even though they are generally the same, there are major variations:

1. Immature crystals
2. Highly geometric crystals
3. Synthetic blood, filaments, polymers
4. Large buddy growth
5. Large various sophisticated crystal biology.

So what is causing the difference?

1. pH ?
2. temperature ?
3. Method of pH transition
4. Nutrients
5. Concentration Level (recall that $C_f = 3$ here on TSP 01)

The one thing that you are recalling is that the pH of TSP 01 was fermented @ 6.0, even though when synth blood was obtained the pH was recorded was ~7.2. This could be quite important.

I want to start testing the idea as soon as is possible - it will be a while before then is available, including the question of whether starch is beneficial or not (when you it seems to be).

Today we need to
1. inspect the cultures
2. Set up the next culture
3. Anesthetic review

Let's split up TSP 12 - WS-A into two parts
Mass of vessel = 357 gms
Mass w/ TSP12 WS-A = 597 Δ = 240 gms
½ = 120 gms.
Mass of smaller vessel = 220 gms 220 + 120 = 340 gms
Set small vessel to Σ of 340 gms

Now, the first big surprise is that the pH of TSP WS-B is not 7.40 as expected. It is 7.11. I did not expect this. It means that it changed more than expected. This means that more attention is going to need to be given to monitoring pH stability, and or we spurious buffering down the road, who is the plan either way.

So let's set 1/2 to pH 6.8 and then start monitoring the culture more closely

—

OK, the next surprise. TSP-12 WS-B, the other 1/2 has a pH of 7.12 and I thought it was 7.40. So we will adjust to 7.40. This was 1 drop of NaOH-KOH so we can see how overly sensitive the systems are. Constant monitoring will now be required.

—

Now TSP 11 needs to be treated very carefully. You can not use the stirrer or disturb it! You would destroy your crystals.

So w/ existing a productive culture, be very careful how you monitor and measure pH.

OK! We have a very important discovery here. TSP 11, what has become a very important and unusual culture, now has a pH of 6.60. We were under the presumption that the pH was 7.24 as it was last established.

The means that it has very recently changed over the last week and that it has had an important, unusual and unexpected impact. This is really important, it shows that the cultures are extremely sensitive to pH changes.

Be very careful not to disrupt or damage these cultures!

Same thing w/ TSP 01. The pH is now 7.80! It started out on 09/20 @ 7.4, on 09/22 it had risen to 7.6. I then readjusted to 7.34 on 09/22 and now it has increased again to 7.8!

So this is really interesting. In one case (TSP 11) the culture became more acidic and caused an entirely different growth form, very unusual.

In the other case, TSP 01, the original culture that eventually resulted in synth blood production, I believe the pH was set @ 6.8 to begin with, now quite some time ago. So it, on the other hand, even with a reset to biological pH, is showing a steady increase in pH.

So obviously a question is, what is causing
? * the difference and opposition in pH of the culture w/ correspondingly significant difference in growth behavior.

Another critical question is, does TSP 01 still contain synth blood? It is now outside of biological pH range.

So another important pH finding. TSP 01, w/a relatively high pH of 7.8 still has the synth blood intact. In addition, there is a very high concentration of CDB. No protein complexes, no crystals. It also seems to have a high polymer nature, cover slip is sticky,

There is therefore a fundamental difference between the two cultures, TSP 11 @ 6.60 w/ sophisticated crystal biology and TSP 01 @ 7.80 w/ synth blood and high levels of slantalone CDB.

This is a great start, Next we need to be able to ~~pro~~ reproduce both situations. The synth blood production, @ this point, remains elusive. I rather recall that the synth blood first appeared @ pH 7.2, which falls between the two cases.

You still have the question of whether the synth blood can be propagated to another culture. Two max factors: 1. pH requirement
2. Temp requirement?
This for incubation @ 98.6 does not appear beneficial.

Next we inspect the TSP 12 w/ starch WS incubated samples.

We also already see the VOCs coming in on the
* new culture. 3 liters, 60 ml sugar, 60 ml fertilizer, 30 ml starch, and .62 ml pink salt. 100°F to be used now. VOC monitoring

TSP 12 WS-A Incubated 98.6
(w/starch - A test)

Surface layer: Short chain COB, COB, & small protein complexes. Nothing remarkable here. No Crystals

The pH is 6.4. I believe that it set at 6.8 so it has drifted slightly acidic.

Bottom layer: Protein complexes, COB.
No Crystal forming.

@ this point, incubation does seem to offer no advantage to accelerate crystallization.

Now we look @ TSP-12 WS-B
(w/starch, incubated, synth blood added)
High protein complex formation, minor crystal presence, no synth blood production or increase known. Very high COB Concentration.

Neither of these culture offer any real advantage @ the time. You target is to identify those conditions that produced the synth blood and that produced biological crystals.

pH of the incubated trial is 6.9.
~~Therefore essentially the same as~~ The also represents a drop relative to initial establishment of 7.40 for TSP 12 WS

Next we look @ TSP 13 - NS No Starch.
Actually I see a little bit of surface activity on the small surplus 30 ml sample.
That is interesting.

Ok, we definitely have significant biocrystal activity. So far only on the smaller space sample - not sure why - but definitely active.
This is a no starch culture.

Now TSP11 where this happens is a starch culture
It has a pH of 6.60

Therefore we conclude that starch or no starch did not impact the development of the Crystals @ an approx pH of 6.8 to 6.8.

This is very useful information.

Now what is needed is to separate TSP 13 larger sample into two categories.
One is to have a pH of 6.7
the other is to have a pH of 7.4

Then you let these set and observe.

The starch was not the issue for biocrystals.
* pH look like it is very sensitive in the pH 6.7 range.

Synth blood circumstances remain unknown.
1. No starch believed
2. pH estimated @ 7.2
* 3. 2 weeks of time
4. Colony successful still @ a pH of 7.8 after already existing

I think that I have reduced the VOCs considerably by having a proper vessel (3 Liters) with a good pressure release lid). VOC's are showing up at 25 ppb but I am not even sensing them even w/ window mostly closed from rain and temperature control. Look, like a big improvement and better insulated as well.

—

Let's think about this: TSP01 & TSP11 are two different outcomes. TSP11 has now been replicated, and the is good. But not the case w/ TSP01.

Recall what happened here. The solution was clear and then most unusual geometric crystals appeared at the bottom. 2 weeks later a blood polymer appeared on the surface. The pH when tested was 7.2.

The fact that it was clear tells us that we did not include the precipitate as we are now. I also do not think I pelleted. This means the solution was decanted off the precipitation.

Precipitation was taking place @ 6.8.
Therefore the main question seems to be if starch was used.

Sep 19 the synth blood was clearly present.
Culture began on ~~Aug 19~~. Sep 19.
Two week prior to Sep 19 was on Sep 05.
Aug 21 I work w/ a potato culture,
I then dismiss it and go back to original.
Precipitation and titration are then used.
Aug 28 TSP is used for the first time
I take pH up to 10.5. I am working w/
enzymes, titration & determination of pI

I centrifuge my solution and separate
off & pure layer of CDB on the bottom.

I mention a TSP @ pH 7.0,
Sep 02 pI analyses is in ~~place~~ active.
We mention @ TSP @ pH 6.5
* Sep 06 Cry ~~stalline~~ geometry is noted
@ pH 6.8
Starch use is mentioned on Sep 12.

Sep 06 date of crystalline geometry matches perfectly
I think that our conditions are

1. 1700 ml H_2O
2. tbs suga, 2 tbs fertilizer
3. mina salt.
4. pH 6.8 to 7.0
5. No starch
6. Decant TSP, not filter after precipitation
7. Crystals w/ in 40 hrs, synth blood in 2 weeks.

So focus on reproducing crystals. Changes in temperature could be an issue.

I have increased production even more to ramp up the pace of trials. I have added the 1700 ml glass ware on top of the 3L pot.
Bottom culture w/ starch, top culture without.

Oct 01 2023

Well, the idea that the veinous crystal biology only takes place @ a lower pH (eg 6.8 vs 7.4) just got blown out of the water. The 30 ml separated sample of TSP 13 - NS No Starch has veinous crystal biology @ a pH of 7.32

This is an enzyme enigma since the original volume sample of TSP-13 NS has NO crystals visible on the surface and it has a pH of 7.4.

This is tricky to establish the controls on the culture.

An observation for today involves TSP-12 WS-A It has a pH of 7.4. It has a gas being released in low quantity.

TSP12-WS-B does not show this.
Also notice that all TSP 12 WS solutions, both A & B are cloudy in color, both w/ pH 6.8 and pH 7.4. Therefore for some reason these solutions do not settled out clear. It is likely because pI seems to be about pH 10.2.

TSP12 - WSA pH 7.4 has extremely high CDB production.

1. CDB (dense)
2. Protein complex (moderate)
3. Smaller crystals but CDB developed.
4. Producing a gas.

TSP13 - NS (no starch) still has a biological crystal layer on top of 30 ml separated sample only.

TSP 13 - NS - no starch - main volume has a very significant crystal layer on the bottom - not veinous. This solution is clear however after precipitation, unlike the TSP 12 series w/ starch. Therefore the starch is having an effect, appears to be that of producing massive CDB numbers in a cloudy solution. (pH 6.8 or 7.40)

TSP13 - No starch, however, is producing a clear solution (pH 7.45) w/ a strong clear layer of crystals on the bottom. This variety actually bears closer resemblance to TSP01 than we have seen for a white. Need, however, strong geometric crystal form and eventual synth blood to match TSP01.

We are, therefore, starting to see a certain level of repetition & identifiable properties of the various cultures. Factor seem to be:

1. pH
2. Starch or no starch
3. Bottom or surface crystal development
4. Decanting vs Filtering post precipitation @ pH of ~6.8
5. Concentration Level? TSP01 is 3X!

Crystals are consistently being produced by:

(1) * no filtering post 6.6 precipitation
one directional pH transition. Surface Crystals have appeared in both starch & non starch solutions, with a pH range from 6.8 to 7.4.

Large number of COB production are occurring in TSP 12-A W/ starch So:

(2) * 1. No filtering or decanting post precipitation one way pH transition @ pH 6.6.
2. Starch preferred

TSP 11 no starch, no filtering represents as closest approach to TSP 01 thus far.

1. Cola is a deep orange
2. No starch is present
3. pH is set @ 7.4
4. Solution is transparent, precipitate settles on bottom — ??? vs Crystallization ???
5. Strong crystal layer, biologically but not geometrically developed on the bottom.
6. Need 2 weeks of time to discover if synth blood develops.

(3)

You are therefore making some progress. Three categories of culture are emerging.

—

VOC's are now finally active in upcoming cultures, it took ~24 hrs to develop.

—

Two topics.

1. Buffer study
2. Molecular weight determination of TSP?
3. At some point, you will need to bring in NIR of TSP culture in an effort to further understand differences.

It is curious, though, that TSP 13 – NS small 30 ml vial sample has produced a surface crystal layer but the original larger volume does not. Temperature variation??

The polo to cultures are coming again some day when I establish repeatability w/ the current generation, and I have a source of phosphorus available.

TSP 11 has now shifted and transformed from Vernon highly geometric crystals to irregular Vernon ~~cry~~ crystals and fully developed sophisticated filaments. Notice that the pH in TSP is last reported @ 6.60 on Sep 30, and it was 7.24 Sep 25. Let's check it.

It is now 6.49 on Oct 01. This is showing a clear trend of increasing acidity as the culture progresses: therefore it is generating acid as it develops further.

you see that the easiest way to transfer the surface crystals to a slide is to dip a needle in and then rinse the needle w/ a drop onto the slide. The act of pipetting the crystals does not seem to be effective. Surface tension is playing a role here.

The culture work in general is showing a very high sensitivity to pH and pH changes.

Oct 02 2023

I inspected blood last night. It looks the ~~best best~~ best as long as I can remember. The CDB ~~as~~ exist exterior to the cells but are not showing up in the interior or attached to the perimeter. Even the central cellular CDB does not seem to be present. Coagulation effects seem to be dramatically reduced. A dried slide prepared easily and smoothly. Results indicate the enzyme strategy may be highly effective w/ in a 2-3 week period.

Here is an interesting question? Was the enzyme impact upon the protein discovered prior to, during, or after precipitation discovery and application? That will be traced down.

—

Next culture series is coming along well. No VOCs @ this time but will let things warm up more.

—

TSP12 - WS-B is showing a very fine surface layer forming - it does not appear crystalline yet. Will look under scope,

Let's look @ surface area ratio of anesthetic study. Assume 10 infant colony structures of 10 um diam on slide of 20 m²

Area $K = \pi r^2 = \pi(\cancel{10-6}\ 5E-6\,m)^2 = \cancel{7.535E-5}$

$= 7.85E-11 \cdot (10) = 7.85E-10\ m^2$ colony

Cover slip surface area $= (20E-3\,mm)^2 = \cancel{4E-2}\ 4E-4\,m^2$

$\frac{4E-4\,m^2}{7.85E-10\,m^2} = 5.10E5$

$= 510{,}000. \quad \frac{1}{510{,}000} = \frac{x}{1{,}000{,}000}$

$x \approx 2$ parts in a million.

DNA testing sensitivity requirement estimate

VOCs are active w/ the cover off the cultures @ 30 ppb to keep it running.

We now have another new form of growth. This brings us up to 12 variations now. This comes from a surface layer growth on TSP-12 WS-B.

pH is last stated on 09/30 as 7.40. Let's measure it. It measures @ pH 6.65. This is a significant change w/in two days.

We are therefore seeing a pattern that the cultures, even when brought up to biological pH of 7.4, steadily transition into an acidic environment. This is expected to place a constant additional stress on the body.

We need to look @ the citrate buffering influence, Urine would be the best case.
How can I make the citrate in - again?

—

Now TSP-12 WSB [Set 1] had been set to biological pH = 7.40

TSP-12 WSB [SET 2] had been set to pH 6.8.

TSP-12 WSB [SET 1] Biological pH 7.4 set is the one that shows the growth form. It looks to be substantial growth developing possibly beneath the surface as well. This is the solution that remains cloudy. The two precipitates carefully one way from 5.8 to 6.8 and then pH delicately was upgraded to 7.40. It always remained cloudy and never settled out as the non-starch cultures do.

The cultures are very good examples of the many significant variations in yeast that are commonly encountered and that show high sensitivity to pH conditions. The make replication considerably more difficult between:

1. Medium strength
2. Level of filtering applied
3. Precipitation effects
4. Temperature effects
5. Concentration effects.
6. Maturity of culture development
7. pH state & pH changes.

The globular clusters of the cultures are heavily insulated from the surrounding CDB/protein medium by a "buffer zone", akin to sac development.

Biological buffers:

1. Citrate
2. Bicarbonate
3. Carnosine — L-histidine is a precursor to Carnosine. B-alanine is a naturally occurring amino acid precursor to Carnosine.

Mitigation strategies now propose
1. Ultrasound
2. Custom balm - balancing act
3. Enzyme strategy
4. Buffery strategies - bicarbonate / enzymes] pancreas

Guess what the pancreas secretes?
1. Digestive enzymes
2. Bicarbonate

Baking soda is "an effective remedy for pancreatis

You can be have too many enzymes and it can induce pancreatis. Think about that.

$\frac{2}{1E6}$ = So one liter = 1E6 uL.
so two PPM = $\frac{2 uL}{1E6 uL}$

DNA test would be required to isolate 2uL out of a liter.

TSP WS-B @ original pH of 7.40
now dropped down to 6.65 is a worthwhile
Culture and deserve escalation.

Elevated Cultures

Culture

TSP01 — are now the elevated

TSP11 — Cultures

TSP12 WS-B w/ starch — Original pH 7.4, now 6.65

TSP13 NS 30ml no starch

Not elevated:

TSP12 WS-B pH at C 6.8

TSP12 WS-A pH at C 7.40

TSP13 No Starch pH at C 7.45

Carbonic acid – Bicarbonate ion

(weak acid) (Conjugate base)

There is the buffer in blood.

$CO_2 + H_2O \rightarrow H_2CO_3 \rightarrow HCO_3^- + H^+$

If the pH of the blood gets too low, the bicarbonate will pick up a H^+ ion and become Carbonic acid. That Carbonic acid will split apart into Carbon Dioxide which is exhaled through the lungs and water.

Three types of enzymes in the pancreas

1. Lipases – to breakdown fats
2. Proteases – to breakdown proteins
3. Amylases to breakdown Carbohydrates

Protease key become the primary target beneficiary.

The target would not be to assist the blood as much as it is to assist the pancreas:

Enzyme 1. the breakdown of proteins w/ protease (a pancreatin, an enzyme)

Acid Neutralization 2. Assist in the neutralization of excess acid production w/ the help of the bicarbonate ion

Both of these are primary functions of the pancreas

Target the problem @ the front end of digestion, not after distributed into the blood

Nutrient absorption are absorbed from the upper small intestine and from there they cross over into the blood.

Pancreas coma BEFORE absorption into the blood.

Yep, hit the target on the front end, not the rear.

soda ash is washy soda is sodium Carbonate
baby soda is sodium bicarbonate.
sodium Carbonate + sodium bi carbonate
makes a buffer.
heavy baby soda will make sodium Carbonate

Oct 03 2023

TSP01 is presenting a surprise. It is surprisingly productive. I had some concern that it was waning or deteriorating. At this point, it is hardly the case.

We have advanced interior biological crystal activity – they seem to be acting as a factory production of the synth blood. It is becoming a more dense culture rather than deteriorating @ this point. It has gone through quite a lot of change since the beginning.

Progression:

2-5 days
1. Clear solution
2. Crystals w/ "immature" development & geometry
   fam on bottom
3. Crystals become very sophisticated and unusual in geometry

2 weeks
4. Red colored network w/ filaments in polymer matrix forms on surface. These were the first synth blood cells seen, basis of paper.
5. Culture appeared as though it might be waning. pH monitoring begins

6. Unsuccessful attempt to transfer to another culture to propagate
7. Density of synth cell gradually increases
8. Crystal biology - apparent synth cell production - recorded today.
9. I believe first pH measured was 7.2 when synth cells observed.

09/20 7.4
09/22 7.6
09/22 ~~7.~~ 7.34 adjusted
09/30 7.8
10/03 7.65 Let's measure.

The latest pH readings very favorable. It tells us what is able to sustain synth blood production. It is a fortunate situation that the pH did not rise more.

—

Since it has not yet been replicated, however, the particular culture funding and situation remain in jeopardy. I am seeking electronically to attempt a repeat, but we are talking 2-3 weeks for every cycle — there is problematic as there are many variables active w/ now 12 different metabolic metabolite products that have developed from just one of the four protein(s) types.

Steps will need to be taken to ~~pot~~ protect and preserve the culture (~60 ml) as long as possible; but also plans to propagate and preserve longer term need to be enacted.

1. Freezing? (how much to sacrifice?)
2. Transfer (To what, exactly?)

I am going to see if my small fridge-freeze
* section will freeze a 5 ml sample.
This culture has become quite valuable.

TSP11 only has a few surface crystals now. This is the most acidic culture @ pH 6.60. I think that it is time to bring it up to 7.4.

I have set it to 7.52 Oct 03

Ok, now for TSP-12 WS-B pH = 7.4 (09/30) however pH dropped to ~ 6.6.
A definite full coverage surface layer has formed on the original full sample

We looked @ this under the scope yesterday. Significant and unusual growth, globular clusters w/in enclosing "sac". This is a case where the pH dropped from ~7.4 to ~~7.6~~ 6.6 as the new surface layer has evolved.

—

The 30 ml sample of TSP-12 WSB now looks to inactive & unproductive, even though it originally was one of the most interesting w/ surface crystals.

—

TSP13 No starch continues to show no activity visible by eye.
It has clean clear sharp immature crystal layer @ bottom. Well continue to monitor. Solution remains clear amber.
pH is now 7.10 vs recorded 7.45, so once again a shift to acidic. Well reset.

—

TSP12 WSA Is starting to show a layer on surface that now consists of iron protein complex, no crystals. pH started @ 7.40. This was the culture w/ the bubble observed developing on the surface.

Now, here is something interesting.

Just yesterday it was stated that the culture had the pH set to 7.40. Understood why the s because the culture was acidic and also did not display much activity other than a few bubbles at surface.

Today the pH reads 6.70 and we have a significant surface layer that shows likely to likely be the iron protein complex. This actually represents a very significant change w/in 24 hrs and now the culture bears closer monitory. It also says that biological activity can take place fairly quickly if the pH is in favorable range. I will test the pH to be 7.4.

I will hold off on JSP-12 WS-B today. pH last recorded @ 6.8.

Ok, let's think about where we are going now:

1. Monitoring of cultures is obviously important.
2. Developing repeatability of the cultures is equally important.
3. Replication of the synthetic blood development would be a significant accomplishment.
15. Magnetic/electromagnetic influence, esp upon crystals?

The previous item develop into a steady state affair.

4. Demonstrating protein interference is obviously what is eventually needed the most. You have made some progress.
   1. Balm - come and go - skin eruption, etc
   2. Ultrasound - also come and go
   3. Enzyme strategy - this needs to be tightened up
   4. pH sensitivity issue and question
      1. Bicarbonate - pancreas relationship
      2. Buffer study - Can you and how would you use buffers? - Pancreas role
      3. Citrate influence?
   5. Inorganic analysis of the protein, whatever you adopt as the standard
   6. Consistent health lineage
   7. Difference and consequently impact of synthetic vs human blood.
   8. Course work.
      Thinkwell, Chemix, Buffer software, Great Courses

Printer Now

   9. Tap into existing IR library - to what purpose?
   10. Other proteins - what are they?
   11. Retrace original earlier culture work - potato influence, agar?
   12. General organic & organic chemistry of the protein

Oct 03 2023

The Centrifuge, at times, is most invaluable. The is the only practical, simple method to purify the "settled" protein. It requires a two stage process, and centrifugation is absolutely required to separate the pure material that is suspected to be of protein character. UV would be first approach here.

1. Centrifuge coarse combined material.
2. Extract top lighter fine layer
3. Centrifuge again mixed in water
4. Separate again w/ minimal water
5. Dry sample (small amount) to desired concentration.

Proceed w/ solubility tests, UV, etc

The term we would like to save some of the original, native TSP, prior to precipitation to biological pH. The is because of buffer exploration anticipated in the future.

I have successfully grown a ~5 ml sample of TSP 01 in the case it can not be replicated easily quickly.

You certainly have a good list of investigation topics. The question is what will be of the greatest value?

Oct 04 2023

Clearly much of what is going on w/ the ~~pro~~ protein(s) is based upon acid-base chemistry. I think it behooves you to understand this more deeply along w/ buffering.

1. Determining the identity of the acid beyond doubt (eg phosphoric acid) would seem to be rather critical. Titration.

2. Understanding buffering could be equally valuable and important.

3. Developing an acid-base mitigation strategy (eg - bicarbonate - pancreas support) would be of much potential value, along w/ enzymes. (Citrate?)

4. Buying NIR and electrochemistry capability directly into the lab would be helpful. (Organic, inorganic analysis of anything under study)

You will have guide the Complementary lab:

1. Difference between synthetic & human blood (while you can't)

2. Oxygen (a earlier culture work) w/ $FeSO_4$, Potash, etc (the cold protein?)

3. Molecular weight determination

4. Health lineage consistency

5. Fantastic coursework available
   1. Thinkwell — Chemistry & Biology
   2. Coursera — synth biology
   3. Duke
   4. Chemix, Buffer software, Chemlab
   5. Vernier experiments, Biology book?

Oct 06 2023

I now need to be more efficient w/ culture time and space used. I like petri dishes now so that should help. Also w/ mitigation trials.

What are some of the trials involved?

1. Citrate
2. Bicarbonate
3. Vit B
4. Enzyme
5. pH
6. Temperature - Incubation
7. Precipitated or not
8. Buffers
9. Starch, No starch
10. $FeSO_4$, Potato
11. Blood nitrate?
12. Concentration?

B Water Series Tests

TSP 40 Series III PPT

1. Buffer development & study
2. Titration in depth
   Acid identification
3. MW determination
4. NIR & Electrochemistry

Oct 07 2023

I now have 4 cultures that can be inoculated & 80-100°F & I watch the temperature carefully until I acquire an incubator. I am getting these set up for multiple simultaneous trials, on the order of two dozen or so. Any idea become easier & fast now w/ smaller volumes required. You will have multiple series. For now to start:

	(NS)		
TSP 20+	No starch, no precip	pH ~ 5.7	
TSP 30+	NS, precip	pH ~ 6.8	
TSP 40+	With Starch (WS), precip	pH ~ 6.8	

4 Incubated trials are available

Native culture material:

TSP 20 Series	NS, No precip	pH ~ 5.7	~125 ml
TSP 30 Series	NS, Precip	pH ~ 6.8	~125 ml
TSP 40 Series	Starch, precip	pH ~ 6.8	~250 ml

First stage is to assess status of existing cultures.

TSP 40 series, ~250 ml shows interesting promise over over 24 hrs. Let's see...

TSP 40 series has a little bit of several forms on the bottom layer appearing.

1. Protein Complex
2. CDB
3. CDB Chains
4. & monitor undeveloped crystals
5. About 1/2 dozen circular structures, possibly synth blood but would need much higher concentration & confirmation.

TSP 40 series (~250 ml, pH 6.8) Starch
Now let's create 2 culture & begin to incubate 75-100°F.
One as is, w/ pH 6.8
Another @ pH ~ 7.4

Next look @ TSP 30 series ~125 ml, precip, pH ~6.8
We see both a surface layer and a bottom layer.
No Starch

Ok, TSP 30 series does have more advanced crystal structure than TSP 40 series. We do have internal reinous structure already developing here as well as more orderly geometry. This is a no starch, precip, pH ~ 6.8 culture

Now let's look @ the bottom layer.
TSP 30 has a productive crystal - protein complex layer. The crystal geometry is not particularly well developed. There is no synth blood hint of any kind.

We could create an incubated culture of this.

One thing that is of interest is that TSP series solution is very clear. ~~They~~ This part does seem to be similar to how TSP behaved but the crystals are not similar in geometry.