CI LABORATORY NOTEBOOK

VOL XVII

Mar 2022 –

Mar 26 2022

I will just start a new volume series Vol 26 stands in its own right.

Recalling the examination of the Coagulation rate of blood when placed in glycerin, it really did not seem to make any real difference.

Although the rate of movement is retarded, it did not make any appreciable difference on the net result of coagulation. The experiment has no special value @ the moment.

I can return to the experiments w/ a saline solution @ a later point.

The objective now is to run additional controls w.r.t. the identification of the existence of a soluble protein via Visible Light Spectrometry.

The first trial w/ be with Collagen ppt powder dissolved in H_2O.

Recovery w/ the red dye reagent. Moderate Concentration
50 ul Collagen Solution

Red Dye	
λ (Peak)	Abs
466.9	.521
496.4	.522
513.7	.506
531.1	.428

514 / 496 = .506 / .522 = Ø.969

531.1 Almost Discernible

When inserting the 50ml of collagen solution I was able to visually detect the change in color to a more purple color in the stream of the injection as it diffuses.

You have also learned from previous trials that you need to recalibrate the spectrometer for each trial.

Collagen

5min

λ	Abs	
494.8	.603	514/495 Ratio = .619/.603 = 1.026
513.7	.619	Visibly more purple also.
530.3	.564	Very identifiable Slope break

Once again, an additional protein control proves the point.

I now have 3 protein control trials all with the same result.

These include
1. Powdered milk
2. Hemoglobin
3. Collagen powder

With the reagent developed, the following are characteristics of protein detection, in order of priority:

1. The ~514/~495 absorbance ratio.

   In general, the ratio will increase w.r.t. to the control of the red dye reagent. It will approach a exceed 1.0 but the increase in the ratio of the relative absorbance peaks is a positive identifying feature. This reflects itself in a shift in color of the reagent to a more purple shade.

2. The increase in the inflection point or minor peak emergence @ ~531 nm is another identifying feature.

3. Visually, the shift in color from the red dye to a more purplish tint is another identifying feature depends upon significant concentration.

One test here used 50 µl of a moderate concentration of collagen powder into a 3 ml cuvette
This ratio is $\frac{3E-3}{50E-6} = 1/60$

The controls are sufficient and the existence of a foreign protein in the blood in significant amounts remains valid.

Mar 27 2022

A brief discussion on the acquisition of Copper Sulfate $CuSO_4$. This chemical compound was absolutely critical and essential to the development of the protein reagent.

Acquiring $CuSO_4$ in the general consumer marketplace is not so straightforward as most raw chemicals of interest are being removed from the shelf & altered to be generally less effective.
There are no aquarium products or pesticide products @ Walmart any more that contain $CuSO_4$ so that made the search more difficult.

I prefer to have independent redundant sourcing for most any item needed if possible.

Another alternative (not the last) was to see if I might be able to make my own $CuSO_4$. In theory I should be able to under the reaction

$Cu + H_2SO_4 \Rightarrow CuSO_4 + 2H_2O + \cdots$

So I did my best to acquire sulfuric acid as a drain cleaner, but I noticed at purchase what is sold now as "deactivated". Sounds like an oxymoron, doesn't it? And it looks like it is...

Along the route of investigation I placed some pure Cu wire into my "sulfuric acid".

No reaction ensues.

I then find that concentrated H_2SO_4 & Cu is supposed to produce a $CuSO_4$ reaction. Dilute H_2SO_4 is not supposed to have a reaction because of the activity series of metals.

I find no reaction of either kind, either dilute or concentrated.

I do notice when H_2O is added to Conc. H_2SO_4 a lot of heat is created, hence the adage of adding acid to water vis versa v.versa.

So now I explain the lack of reaction from Concentrated H_2SO_4. Research the "deactivation" that is now incorporated into "Consumer" H_2SO_4 the process is exactly the problem - it reduces the reaction with metal (think plumbing and pipes). Therefore the modified H_2SO_4 defeats the very objective I had in mind i.e, the reaction w/ metals. This is my last application for the failure.

So producing my own $CuSO_4$ on side was a BUST.

I had to resort to my last alternative, which I already knew ahead of all this from my experience. Thank goodness for a decent hardware store.

Stump Remover is pure $CuSO_4$. It is the perfect solution and thus far is my only method to allow production of my protein reagent.

And, as we have seen, use of the protein reagent is a critical and capstone element of all CI research over the last 25 years.

Therefore what might seem an uninteresting and unimportant topic can, in reality, be very important.

Stroop removed has made all that is known now possible. Access to reagents and useful chemicals has become increasingly difficult through the years. Drug user abuse is often cited as the rationale to eliminate the study and use of chemistry in the general population.

April 01 2022

There are two issues worthy of investigation

1. Protein Concentration
2. Magnetism
3. Protein Characterization - foam prep

Your best avenue on protein concentration is probably going to be w/ NIR. It may be of interest w/ VIS spectroscopy but I suspect it would be difficult to be sensitive enough.

W.r.t. magnetism you need to decide what is your best sample material. At the point it would seem to be the foam prep if you can gather sufficient material.

Between the two issues, magnetism questions are probably of greater importance. Protein concentration levels would be of a relative nature only most likely. Protein concentration would be valuable for method development but is unlikely to produce any essential result right now, especially w/ the minimal equipment available.

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Now for another topic. As far as the foam protein characteristics, the most useful project would be that of electrochemical characterization. You can now apply this to the rotating system. You would have both NIR and electrochemical identification.

Apr 06 2022

I have a fresh blood sample to work; probably 3-5 drops in ~~3-5~~ 3 ml of distilled water.

It can be seen that the black electrode is again immediately active and foam material is already beginning to accumulate on the surface of the tubular Al electrode.

We will have similar goals in the place of the research.

1. The first is to collect the foam precip material as a distinct entity.

2. We will carefully observe the dynamics of the blood also along the way. Surprisingly the redox reaction are very quiet on the first two runs but the foam precip is started immediately. Also be aware that I am using brand new electrodes.

3. After 5 scans, there is definitely activity taking place but it is of fairly low magnitude, ie ~ 0.15 mA instead of 2-3 mA.

4. Next observation is that the foam precip starts out as pure white Fe color, but over time the foam precip is taking on a more brownish, dull tint.

5. After about 1 scans, the blood is slowing likely to be settling in the bottom of the tube. There is still gas bubbles produced from the black electrode. So one thing the means is that the end of the electrode is now closer to or making contact w/ the settled blood.

Now the next thing to observe @ this stage is that the electrochemical activity has diminished in magnitude. The foam preps seem to be gradually become more dense but not any greater in volume. The main solution is gradually becoming more transparent.

6. Now after 11 scans the foam prep is now starting to seep over the rim of the tube. There is something it will be looking for. It could be that the rate of foam prep production increases after the layers of the vial have fully formed and are separated. The 3 layers are

1. The foam-prep on the top
2. The bluish solution which becomes quite clear and transparent, but with a light reddish brown tint
3. The bright red blood conglomerate that has settled to the bottom of the tube.

7. Visually, after a dozen scans, the results of the vial are identical to the previous runs. However, on the run that far any electrochemical redox behavior is much less dynamic and lower in magnitude than the past runs. I am not sure what that means yet.

8. Now is when the black electrode actually seems to increase the rate of foam precip production. An increase of generation of foam-precip can start to be seen on the surface of the electrode as it then rises to the surface. After about 5 runs of no redox reaction already taking place, redox activity now shows itself to be increasing again, although the magnitude again remains low, eg 0.10 mA.

However, foam-precip production is decidedly now increasing @ this point ~12 scans in. This behavior has been observed before.

The time I will be transferring the foam precip to a 5ml beaker in preparation for further analyses.

9. The most productive method of collecting the foam-precip is with a scoop, not an eye dropper.

10. As suspected, the water volume in the vial is slowly decreasing as the foam precip is formed.

11. It is also noticed that there is some blood Color mixed within the foam precip but the vast majority of the blood Odor remains settled on the bottom of the tube.

12. In addition, as also noticed before, the transitional stage in the overall reaction process does change the Color of the vial solution to a greenish brown vs the less earlier reddish brown. The indicate a significant reaction likely involving iron that is now Converted to a Fe+2 state.

13. Second sequence of foam precip now taking place. Again starting with a Color solution in vial remains a a light yellow green tint.

14. The foam collection of the first layer is gradually transforming to the liquid as it settles, much like soap suds would. It is actually a fairly dark reddish color, darker than the blood remaining @ the bottom of the vial. The settled blood is now almost a light red Color, not as vivid.

15. After significant time, e.g. 18 seconds, there is a very clean separation takes place in the vial.

1. A lighter red layer of blood conglomerate @ the bottom of the tube.

2. A light green yellow green tint to a now very clear solution comprising the bulk of the vial, which started out as distilled water. Suggestions are that we have an Fe^{+2} ion in solution.

3. Foam precip layer on the top.

Clearly a separation process to take place.

4. You are now centrifuging the foam precipitate. Three layers are again showing up.

1. What appear once again to be filamentous materials on the surface orange red

2 A light ~~yellow green~~ tint, ~~definitely not blood colored~~

3. A very small mass of solids on the bottom.

Sample materials are slight in mass except for green tint solution - further analyses will present its difficulties.

16. The settled blood in the prong tube is extremely bright red. Defenately a distinction from the foam.

17. Our focus is now going to be the application of ACV upon the layers of the foam precip, dissolved in NaOH by non soluble.

OK, the first thing that I am going to do is study the ~~effect~~ ACV behavior of the foam precipitate base solution. Then these extracted by pipette from the center of the centrifuged test tube.

I still have the remaining blood vial on hold, and it is not yet centrifuged.

Now I have noticed something interest. When the remain of the foam ppt precip test tube are restored to mix all 3 layers again for a repeat centrifuge

FOAM is again produced. This foam then is therefore a crucial element of composition of the contents. There are still indeed many question about the nature of this "foam" aspect of solution.

I am going to start @ a macro level and run from −3.5 to +3.5 and then alternate.

There is a very dominant peak @ −2.78V
Na, K, or Ca are likely candidates here

There is additional activity lower, such as the region −3.3V. A scaled view of the plot will be needed

There is a small amount of foam likely produced again @ the black electrode. In addition, even though there was clear centrifuged lead solution, it appears that solid or more dense material, tan in color, is forming at the new vial now they subjected to ACV. There appears to be an additional separation that may be in place.

There looks to be quite significant. After a single ACV run from −3.5 to 3.5 plus a run from +3.5 to −3.5 there is the formation of dense solid material, tan in color, at the bottom of the vial. Recall the were centrifuged and transparent lead solution

*

The now say that we have an iterative process in place that is now clearing a previously unseen material. The steps now are

1. ACV applied to fresh blood in H_2O
2. Acquire foam sprays after sufficient ACV application
3. Centrifuge the foam sprays which have been solubilized in methanol H_2O
4. Extract the clear blue solution from the centrifuged tube
5. Expand this extraction to additional ACV sample full cycle of ± -3.5 to 3.5

6. Examine the solid material that precipitate out @ the bottom of the vial with
   1. Microscopy
   2. Protein extraction
   3. NIR if sufficient material exists

to for we notice peak @ -3.3V NH_3?
-2.78V Na, Ca, K?
-.76

∴ To Could easily be $Fe^{+2} \rightarrow Fe^{3+} + e^-$
the would explain the tan color that looks like rust.

But you know that the could easily be accompanied with CDB a protein once dissolved in lye.

The tells us that we likely extracted Fe^{+2} in the foam - prey product phase and that there was biogenesis up to and through the carbafuge process.

Then it says we are converting that to Fe^{3+} through additional ACV.

The Fe^{+3} would then likely form an oxide Fe_2O_3 The would be ferric oxide.

The vial we have all color and the tan solid settle to the bottom. The solid will need to be evaluated later to see.

After 4 rounds of -3.5 to 0:
Foam production is more minimal
ACV activity continues to decrease
Toilet-Clean up
Tan solid mass settle to vial bottom.

Apr 07 2022

OK, it is not just an "oxide"

We have developed a very effective means of isolating the CDB - developing filaments, and almost certain to be determined protein structure.

This accomplished with a two-stage process of AC voltammetry combined w/ centrifugation.

The general method outlined as follows is:

1. A min. of a couple of drops of fresh blood in a vial of ~ 3 mL of distilled water appears to be an adequate and viable sample. More blood, e.g. 5-10 drops would probably just make the process a little easier and more dependable production.

2. Subject the blood to ACV Voltammetry under the conditions mentioned earlier (other combinations may also work, of course)

E Cond 4.0V
t Cond 5 sec
E begin -3.5V
E end 0V
E step .002V
E ac .02V
Freq 100 Hz
Scan Rate .01V/s

Each scan takes ~ 6 min
~20 scans are required until the first separation is complete, ie ~2 hrs.

3. Three primary layers will form:

1. A foam precipitate on the surface of the solution. This is to be accumulated, isolated and transferred to separate holding for additional analysis

2. A blue solution which occupies the bulk of the vial. This will have a tint to it, eg orange-red -

3. What most certainly appears to be a blood conglomerate that eventually settles out of solution to rest on the bottom. The substance is wet red in color and is the residual blood conglomerate.

Several stages of clarity and electrode activity will occur over the 2 hour period.

The primary target here is the isolation of the foam precipitate that forms on the surface of the vial subjected to ACV.

4. The next primary stage is to apply centrifugation to the foam-precipitate. Take the foam precipitate and mix it with ~3-5 mL of H_2O.

Apply the centrifuge. Three layers will form:

1. A light floaty layer, by previous examination shown to be largely the presence of lightly developed filament material.

2. A base solution that occupies the majority of the vial. It will be clear & transparent with a tint of color. Depending upon the purity & excretion from blood traces in the foam precip it can be orange-green & or yellow green tint.

3. Some solids (small amount) will be on the bottom. This can easily be a Complex Combination of both residual blood cells & CDB.

5. The advancement of work in the series enables the further purification and separation & isolation that take place with a second titration of ACV. The ACV is applied to the clear & transparent solution that results from previous centrifugation. Nothing is visible to the naked eye between the layers and the clear solution and it appears to be quite pure.

6. However, upon application of the second gelation of ACV (same Gobbs conditions as before) (I did have one scan, however, that ran from +3.5V to −3.5V, in reverse order).

it will be noticed that an additional precipitate falls out of solution.

It will be uniform in color, of tan appearance, and it will settle to the bottom of the electrochemical vial.

Thus far the appearance of a quite pure isolate.

7. Now the final level of clinching the result here is inspection under the microscope. The result is quite classic, and indeed very pure in nature.

1. CDB w/out doubt, in massive numbers. A highly effective purification method that requires ~4 hrs to complete the 2 sets of ACV scans.

2. The origin of filament production is also quite clear here. This is identical to that recorded in earlier research papers

3. And lastly, the final word will come from the protein detection methods that have been developed.

All signs are, however, that the dominant component present, in addition to that of the CDB and occasional developing filaments (sample is now 24 hrs old now)

is the almost certain existence of a protein.

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Before proceeding further, the next logical step will be to apply the protein detection reagent & VIS spectrometry to the critical question.

Having my precipitate isolated & conduct the protein test is a legitimate issue but I will proceed on the assumption that it will have sufficient material present.

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Now we will go to protein testing!

We know that the suspected protein are insoluble since they are precipitated out of solution in water. We also know that electrochemical processes are required for the precipitation to occur.

Now, our protein detection method requires that the protein be solubilized in water & be responsive to the dye & reagent.

From experience, we know that a strong alkali (eg KOH, NaOH) can be used to dissolve the protein. I have done so, it does appear as though I have more than sufficient material & conducted the protein test.

As the method & prepare the protein for testing is:

1. Place ~ 1 ml of distilled water in a test tube.

2. Pipette the settled precipitate, already been examined under the microscope as a combination of CDB, oxynaty filaments, and almost certainly protein proteids and transfer it unto ~ 1 ml of H_2O. The ultimately remains insoluble.

3. Now add 1-2 drops of strong alkali to the tube, ie NaOH- / KOH. Estimated at 5-10 Molar.

4. The is remarkably effective. The ~1 ml solution is now of a quick deep olive green color (and I am also quite familiar with from previous research) and appears completely transparent and now fully dissolved.

Protein Testing:

We now commence to VIS spectrometry in conjunction w/ the protein detection reagents.

We will start w/ slight concentrations and amounts of the dissolved precipitate into the reagent.

I have used 50 ul of the concentrate dissolved into ~3 ml of the reagent in a first trial. (60 to 1 ratio)

We have an absolute positive test for the existence of protein w/ the 512/496 ratio.

Our numbers are $\frac{.027}{.026} = 1.038$ or 1/1

We know from extensive previous analysis that any 512/496 ratio that is 1/1 is clear evidence of protein existence.

Our work now has positive identification and isolation of the COB foreign protein in the blood.

$\frac{514}{492}$ ratio, ~ 10 min later is $\frac{.033}{.029} = 1.138$

One thing I am doing here also is improving the sensitivity interpretation of the protein reagent test.

It is more sensible to use the reagent itself as the reference for VIS spectrometry vs water, and the Company reagent, & then for the Company that is reagent + protein.

Using the reagent alone as a reference allows for any change introduced into that reagent, and therefore any spectrum variable becomes important.

The 512/496 is especially and easily apparent in the case.

There are additional absorbance peaks that show up in this method but we know the 512/496 ratio to be more than sufficient.

Let's run another melt control using the method.

Now running a Control w/ milk we find
We find even a more more distinct the
spectrum spread using the reagent & the reference.

The 515/Abs 496 ratio is certainly there
and the magnitude is quite strong $\frac{.009}{.003} = 3 \quad \geq 1$

However the most dramatic absorption peak is @ 565-566nm.
There is a large broad peak.

And this is exactly also what we get w/ the
CB: CDB Concentrate, a very deep broad
peak @ 560 nm.

This is actually a much more sensitive method
yet of identifying the existence of a protein
in addition to the use of the 512/Abs 496
ratio.
I suspect I could detect protein to even
a much lower concentration w/ this method.

Either way the existence of the CDB originating
filaments & the CDB generated protein
have been positively identified by
both visual microscopy and VIS spectrometry.
The protein is foreign and within the
blood samples as examined at length.

X Any blood containing CDB, which thus far is
any and all for 15+ years, logically contains
the foreign protein. We also see that this protein can
be generated via an electrochemical process.

I am not sure that many people will come to understand the significance of the recent work over the past couple of months. I do not anticipate many to know whenever it may be.

The proportional constitution of the protein and the CDB seems, from a visual perspective, quite high. At most I have been working with a few drops of blood. I suspect my ~1 ml of protein-CDB concentrate would be sufficient to conduct 100-200 protein detection tests as no more than 50 ul was required per test and I suspect I could detect with as low as 10 ul.

The protein-CDB existence is almost certainly @ the root of the extensive and serious blood coagulation within literally all samples that have been observed. I have not seen a blood sample since the initial blood work was started that does not bear the presence of the CDB.

What we have therefore is a resident foreign microorganism in the blood in high proportion. It has been demonstrated that the protein can certainly be isolated via electrochemical means. It also appears quite the case that the foreign protein can also actually be generated by the same electromagnetic energy. This may well apply to advanced filament generation

as well. If the latter two cases prove to be true, then is absolutely extraordinary.

The case for this is the volume of protein & filament that is possible after the electromagnetic processing of the blood; such advanced existence in next life propositions has never been observed microscopically w/in the same sample examined.

It is therefore quite apparent that a mechanism of destructive force to blood, and therefore human and mammalia life, can be instituted within a brief period of time. The annihilation of the human race on short notice is conceptually very much in range with the results of the work.

It was certainly never intended to be discovered or known, but it is now our truth.

Of course the development of non harmful interference to the development of their microbiology and subsequent protein expression is desirable, but that is not within my range under current conditions and resources. The need has always been obvious. It is certainly known now how serious the problem is. This work still precedes the COVID era influence, which does remain an objective of my research if I can gain access to adequate & sufficient blood samples.

actually this page is not VOID.
It remains under active scrutiny.
Apr 08 2022 2320 MT

April 08 2022

Another important observation in the more recent blood analysis, for previous scans the current maximums reached in the ACV processing was that of a few to several mA (eg 2-8 mA).

In the most recent run, under highly controlled conditions w/ iterative processing and extraordinary & confirmed results, the current flow observed was most always in the

uA range, even the lower ranges of uA.

This is <u>microamps</u>.

This says that a most negligible amount of current (also coupled with a very low AC signal - 100 Hz) was highly effective @ producing the foam - precipitate separation, ie, the CDB, emerg. ~~rese~~ ... filaments, and the protein in abundance.

This statement remains valid → This a negligible induced signal that has produced a dramatic transformation in the state of the blood. This transformation can only be interpreted as of lethal capacity within a short time period.

This statement remains valid: One of our next questions now is to see if we can determine the polarity of the electrode that produces the separation & precipitation.

Apr 08 2022 (cont)

I am doing some control work w/ experimentation of electrode variation

The issues are:
1. Detection of a broken electrode
2. Electrode scenario 1:
   Work electrode (red)
   Counter electrode (black) (Auxiliary Electrode)
   Ground (green)
3. Electrode scenario 2:
   Work electrode (red)
   Counter electrode (black) (Auxiliary Electrode)
   Reference electrode (blue)

Let's take care of item 1 first.
I noticed yesterday variation and inconsistency in the behavior of the ACV process. During additional investigation today of a salt control sample, I noticed a repeat of these problems w/ intermittent signals & unexpected drops in signal magnitude.

I examined the electrodes and found one to be broken and it has been replaced. This has solved one important problem.

The next question that arises is observation of difference in behavior of data collection between scenarios 1 & 2 above. They may both end up being of value.

The observation @ this point seems to be the following, but the curve is also an active point of enquiry.

Scenario 1 seems to produce sharp distinctive peak data during the scan run. This does seem to be most valuable, but it also shows itself to be very sensitive to dynamic change in the electrochemical environment. This may well also be an advantage.

Scenario 2 seems to produce much smoother curves that change much more gradually over the timed scans. For this case it is simple to monitor the change in the dynamic environment, but it may not provide the depth of data that Scenario 1 does. For this case it may be that subtle peaks and inflection points will be the primary data points of interest?

Two important questions now exist:

1. Do both Scenario 1 & Scenario 2 produce the foam-precipitate, & subsequent CDB-oxidized filaments - particle separation & production?

2. What is the polarity of the foam-prep active electrode?

Those are the questions now under review.

Early signs are that Scenario 2 does not produce the foam precip and that the electrode are not readily as active in this mode. This also corresponds to the smoother data graphs that result. There is, however, still definite electrochemical activity taking place under Scenario 2; just not as dramatic.

I will assess Scenario 2 after a dozen runs & then switch to Scenario 1.
- No. I will switch after 6 runs. I believe I have sufficient data to make the comparison already.

Ok, the notes of opening on Feb 25 2022 settle and confirm the issue, Scenario 1 is what produces the usable data & significant electrode activity.

The broken electrode definitely confused matters as well as the electrode configuration of Feb 22 had been lost.

This is all very helpful. Scenario 2 configuration may actually also have value of a different sort.

On the blood run, the first pass clearly identifies both Na & Ca ions/ions in the blood (-2.81 V & -2.74 V respectively)

One of the big questions that arises, however in the sudden chg's; loss or appearance of various peaks. + Cannot fully explain that.

Our Na & Ca peaks have quite expected magnitudes in the range of 4-7 mA. These are very respectable signal mag. magnitudes.

Also w/in 2 secs, foam-precipitation generation is observed.

Now I am telling that the current flow is actually, under many circumstances commonly as low as 6 UA

that is microamps.

When a redox reaction does proceed readily, such as in the case of Na or Ca within blood, the current spikes up to 4-7 mA, on a factor of ~ 1000 increase.

But the global electrolysis of blood reactions actually involves only a few UA ie MICROAMPS, and it is indeed all that is required to produce the foam precip.

The mean the "VOIDED" page is NOT to be voided.

What is different today is that the general ACV cyclic voltammogram is not nearly as responsive in data collection as earlier runs were. I have no explanation for that right now.

The foam prep reaction continues to occur exactly as before. However the data collection phase is almost a non event. The ~~started~~ today w/ a control salt sample being examined.

What happens is I get a single scan that shows 2 sharp distinct peaks that match Na & Ca - Na & Ca and then the data variation ceases. It is thought the instrument is not nearly as sensitive as before that showed a continuous dynamic chemical redox environment.

As what is going on here? At this point I do not know.

CDB separation is not affected by the situation, it continues exactly as before.

The collection of redox data appears to be the issue and only here. There is no comparison w/ previous runs.

Here is what is happening now. I am regressing on Calibration, reference and control data. I have variations in data collection that I am attempting to understand.

I have hooked up to the Calibration test sensor provided w/ the Palmsens. The Calibration noise sensor test has passed w/ flying colors. I am actually in a very low probable environment.

What does surprise me is the sensitivity of the test to EStep.

The test method starts w/ EStep = .005

The actually generates HIGH NOISE even though the number is small.

If you change EStep to 0.05 you get a smooth wonderful curve

but as a matter of fact your noise floor here is so low that it will get underloaded so you need to lower the range to pA - pico Amps!

But if you change it to 0.1 you get a very Cobalt graph. So it is very sensitive to EStep.

So you can see one why you are often getting a smooth graph and those parameters are not likely to be obvious and will require head and eye coordination

But the lead paste very well.

Now I think that I will investigate Controls w/ Pink salt.

Here are a couple of other important observations:

1. The test sensor - calibration is using Linear Sweep Voltammetry as the method - generally a less sophisticated method not very useful for elemental identification. But a hard solid reference.

2. Of greatest interest to me right now is that Electrode Scenario 2 is being used. This is Connected w/ the smooth graphs collected earlier.

So we need to work w/ salt (general reference only, as it is Pink salt - more Complex) and the electrode scenarios and the electrical parameters to get a greater sense of what we are measuring in the electrochemical environment.

All of these waves are separate from CDB isolation. CDB isolation and protein denaturation appear to depend primarily upon fundamental electrolysis.

Ok, the first Control scans are very valuable.

The graph is tabulating over time, ~ 6 runs.

The first derivative is the most useful since it is a smooth curve. The curves are very repeatable which is very beneficial.

Roughly 5-6 elements were identified fairly easily, e.g. Na, Ca, Mg, Mn, Fe

1st Derivative peaks and zero crossings are your most obvious points of interest.

Your control conditions are

Econd = 4V tcond = 5sec

Ebegin -3.5 Eend 3.5

Estep = .05 Eac = .1 f = 100Hz

Scan rate = .05

Now adjust freq to 500Hz, 1000Hz

The lower freq of 50 Hz may actually have accentuated some of the inflection points and therefore give crossings.

Next, increasing Eac from 0.1 to a max of 0.25 markedly increased the magnitude of Cdirect from 3 to 8 mA

Decreasing Eac from ~~0.5~~ 0.1 to 0.05 does decrease the sensitivity of the curves

Decreasing EStep from .05 to .02 increases the data points & we also know affects the resolution of the graph.

Therefore reasonable steady Condition appear to be w/ Scenario 2 electrodes

-3.5V to +3.5V
EStep = .02
Eac = 0.2
Freq = 50Hz
Scan rate = .05 — .03 (higher res)

Decreasing scan rate increases time of graph

The method does seem to be giving smooth repeatable results

Apr 09 2022

OK I am now investigating the contributions to both AC & DC components of the system

There is a very interesting aspect of the analyses. Each curve has information to offer both independently and collaboratively.

Differential analysis is the most diverse and productive method of element identification

The ways to consider look @ both DC & AC components of the system.

Notice the DC contributions span from ~ 30 to +30 mV

K, Na, Al, Ca, Mg Mn have each been identified with the salt control analyses. The results are all very reasonable.

There are progress has been made with

1. Electrode configuration clarification
2. DC - AC Component analysis
3. Differential analysis

I will also continue to examine Scenario 1 electrode configuration as it is known to produce useful sharp sharp peak data.

Now let's look @ blood again.

It is also important to note that the graphite electrodes do suffer some disruption and deterioration in the process. So some level of graphite is expected in the analysis process.

The blood analysis is indeed shown itself to be equally insightful under these refined conditions.

Both AC & DC components appear to have very useful information contained within them.

Differential analysis should also help give a better view

In addition to AC-DC component analysis, differential analysis, corroborative AC-DC analysis, also where the voltage direction, eg from +3.5 to -3.5V.

Notice you do not have electrode conditioning active; it does not appear to be beneficial.

The blood, as expected, does show itself to be a more dynamic environment than the salt solution does.

Apr 10 2022

I am now looking @ the ACV electrochemical profile of the purified isolated ACV Iterative EDB-Purified protein complex.

I am now taking advantage of the reference & electrode evaluations from the previous session. It was very fruitful as we have very time efficient smooth graphs taking place. Each scan is now 4 minutes of smooth data profile.

I am also finding it extremely beneficial to monitor and record both the AC & DC components of the profile. They both have something to contribute. The DC component actually seems to contribute a greater current magnitude and should generally be easier to integrate for peaks & inflection points.

I am using 50ul of the Concentrated NaOH dissolved protein w/ ~ 3 ml of H_2O. K & Na are therefore already expelled & exist independently and therefore will be disregarded if identified.

Conditions are:

Ebegin −3.5 V End 3.5
Estep 0.02 V
Eac 0.2 V time ~ 4 m
freq = 50 Hz n = 352
Scan Rate = .03 V/s

I believe this file to be: Apr 13 2022 1745 3.5 to -3.5 (Critical positive to negative sequence and also the latest file date stored)

Another Very Important Page
CDB Inorganic Electrochemical Analysis

Na, K, OH already known to be in solute.

Initial peaks & inflection points appearing. Derivative analysis

DC(0) (1) ... (n) AC

+1.38 Cl(1.36) +1.40 Cl(1.46)

+1.47 Cl(1.46)

+1.60

+1.69 Al(1.68)

+1.76 H_2O_2 H_2O(1.78)

+1.81 H_2O_2, H_2O(1.78)

? +1.93 unclear

? +2.13 F(2.17) Mg(2.02) Ni(2.05) Al, F(2.07) N(2.08) O_2,O_3(2.08) SO_4(2.0)

+2.63 Na(2.71)

+2.40 Mg(2.36)

+2.83 +2.84 Ca(2.84)

+2.95 K(2.93)

+3.11 Li(3.04)

+3.29

-0.51 Cu(1.52) I(1.54)

+.56 Cu(1.52) I(1.54)

-2.26 Mg(2.36)

-2.14 Na(2.71) -2.66 Na(2.71)

-2.84 Ca(2.84) -2.89 Ca(2.84)

-2.47 Mg(2.36)

-3.16

2.79 Na(2.74)

-3.34 HN_3(3.34)

[2.22 / -2.22] Mg(2.36) F(2.17)

Strong!

* Strong / Consistent Candidates...

Fl, Cl, I, H_2O_2
Mg, Ca, Al HN_3

and what about I-on? Inquire...

There are extremely plausible findings in conjunction with that which is already known. It says that the halogens are a serious issue along w/ H_2O_2 oxidation and mineral depletion. This alt would explain a great deal.

April 12 2022

Today I work w/ the protein concentrate from -3.5 to 0 V.

~~I maintain to~~ I increase the resolution of the data by a factor of 2 such that N will remain as 352 data points. Therefore I have changed EStep from 0.02V to 0.01V

Conditions are the same

Ebegin -3.5 V
End 0 V
EStep 0.01V
EAC 0.2V
Freq 50 Hz
Scan rate 0.03 V/sec

I also monitor and record BOTH AC & DC components. This is a valuable technique.

Then, once again, present fast smooth data collection. Time for each scan ~ 2 min

In the case the red (working) electrode is the most active w/ most visible w/ gaseous production observed.

April 14 2022 Na & K are excluded from base solute.

Protein data analysis - Continued. Numerous ACV scans. Let's work in reverse order. Standard conditions:
-3.5V to +3.5V EStep .02 Eac Ø.2 f = 50Hz ScanRate .03V/sec N=357

y° ACV	y° DC	y' DC	y' ACV
-3.47	~1.16, 1.15	-.75 3.46	3.46
	~1.58	-3.48	-3.39
-3.35, -3.34	~1.55	-Ø.13	-3.38
-3.29	~3.29	3.28, -3.29	-.61
-3.30	~3.29	3.29	
-3.21, -3.20	~-3.16, 3.22	3.22	
-3.23	~3.18		
	3.48		
-3.04		-3.00	
	HN_3 Nitrogen mustards		
-2.96	Suppress the immune system	-2.96	
		3.05	
-2.89		-2.59	
		-2.81	
		-2.89	
		-2.70	
		-3.36	
		2.61	
		+3.06	
		-2.85	
		2.60	

Ranks:

~~-2.96~~	~~Ø~~
+/- 3.29	1000
+/- 3.47	500
+/- 3.22	455
+/- 3.05	300
- 3.36	238
+ 1.56	95
~~+/- 2.64~~	~~54~~
-.75	Ø
-.61	Ø

Ranking:

			Score $\frac{n}{\sigma^5}$
3.47	5 .010	-3.47, 3.46, 3.48, 3.48, 3.46	500
3.36	5 .021	-3.39, -3.38, -3.36, -3.35, -3.34	238
3.29	6 .006	-3.30, -3.29, 3.29, 3.29, 3.28, -3.29, 3.29	1000
3.22	5 .011	3.22, -3.21, -3.20, -3.23, 3.22	455
3.05	3 .010	3.06, -3.04, 3.05	300
2.96	2 Ø	-2.96, -2.96	∞
2.86	5 .033	-2.89, -2.85 -2.89, -2.85, -2.81	
		-.75	Ø
		-.61	Ø
~~1.58~~	2 .021	1.58, 1.55	95
2.64	3 .055	-2.70, 2.61, 2.60	54

We are now analyzing a scan from -3.5 to 3.5V

K & Na are excluded from results due to have solute for protein.

We have candidates ranked (all are extremely likely). Dr. L think that are real peaks should be on the order of $\left(\frac{n}{05}\right) + 50$

since even a single entry indicates a fairly likely chance of existence.

Our current real set scores are therefore

Score		
~~∞~~	~~-2.96~~ ~~K~~	Negate -2.94 $CaSO_4$
1050	+/-3.29	3.34 N_2, HN_3 Nitrogen mustards, serious issue suppresses immune system.
550	+/- 3.41	None?
505	+/- 3.22	None, HN_3, N_2?
350	+/- 3.05	Cs^+ 3.03 Li 3.04
288	-3.36	N_2, HN_3
		NO 1.59 HIO_3 1.57 MnO_4 1.56
145	+1.56	Al^{3+} 1.68 $HClO_2$ 1.67 HClO 1.63 Ti^{2+} 1.60
~~104~~	~~+/-2.64~~ ~~Na~~	Negate Br_2 1.58, 1.57 IO_3^- 1.59
50	-.75	Se Ø.74 SO_4 Ø.75 Zn -Ø.76 Br Ø.77
		Fe^{+3}, Fe^{2+} Ø.77 Ca Ø.78 C_2H_2 Ø.73 ⬡ Ø.73
50	-.61	Cr, Co FeOH Ø.77 Fe^{2+} Ø.74 Ni
.5[illegible].61		MnO_4 Ø.60 NO_3 Ø.80
X<.56		SO_3 -Ø.66 Ø.56 MnO_4 SO_3 -Ø.57 Cd -Ø.57 CH_4 Ø.58
		(Br -Ø.61) Cd -Ø.61 Cu -Ø.64 I Ø.62 H_2SO_3 Ø.57 (I_2 Ø.54 Ø.54)

It seems that our most likely isotopes or elements/ scan are:

		Actual
1.	+- 3.29 N_2, HN_3	3.34
	- 3.36	

2. Fe & Halogen (Br, I, Cl possible)

Other Candidates w/ Consideration
SO_4, SO_3, Mn

My Analysis on Apr 10 2022 led to the following assessment.

HN_3 Unexplained: ±3.47 HN_3 OK
H_2O_2 K(2.93) OK ±3.05 Cs 3.03 Li 3.04
Mg, Ca, Al +1.93
Cl, I, Fl

The combination now leads to the following candidates.

HN_3	Nitrogen Mustards	suppression of immune
H_2O_2	Peroxide	oxidation
Mg Ca, Al, Fe	Elements	replacement, interruption
Cl, I, Fl, Br	Halogens	major substitution, toxicity issues

We love this for examined sequence of -3.5V to +3.5

File Name is:
LR APR 14 2022 1030 Exclude Na & K
Next sequence is also -3.5 to 3.5V

DC 4°	DC 4'	AC 4°	AC 4'
-3.35 ✓			✓ -3.40 -3.43 -3.41
			✓ -3.21
	-2.33 ✓		-3.03 -3.04
			-2.61 -2.69 -2.57 -2.56
		-1.87 ✓	-1.85 ✓
-1.54 ✓			-2.36 ✓
	-0.28 ✓		-.25 -.24
		-0.12 ✓	
+1.43		+.58 ✓	
	2.08 ✓	+1.82 ✓	+1.79 ✓
			+2.08 ✓
			2.78
			+2.29 ✓ 2.29 ✓
			2.93
		+2.99 ✓	3.03 ✓ 3.03 ✓
	3.26 ✓		+3.26 ✓
			3.33 ✓ 3.31 ✓
	3.44 ✓		3.43 ✓

Notable locations are:

3.43, 3.44 −3.35, −3.40, −3.43, −3.41 $\bar{X} = 3.42$ ✓ $\sigma_s = .041$

−2.61, −2.69, −2.57, −2.56 $\bar{X} = 2.61$ ✓ $\sigma_s = .059$

−2.33, −2.36, 2.29, 2.29 $\bar{X} = 2.32$ ✓ $\sigma_s = .034$

−3.03, −3.04, 2.99, 3.03, 3.03 $\bar{X} = 3.02$ ✓ $\sigma_s = .019$

3.26, 3.26, 3.33, 3.31, −3.21 $\bar{X} = 3.26$ ✓ $\sigma_s = .058$

1.82, 1.79, 1.87, 1.85 $\bar{X} = 1.83$ $\sigma_s = .035$

2.08 F (2.17) Li (2.20) AlF (2.07) Mg (2.02)

Ø.26 SO_4 (0.17)

Strays:

−1.54 $\bar{X}$ of 1.54 & 1.43 = 1.48 Cl^- (1.46)

−.12 SO_4 (.17)

.58 We also have Ø.51, Ø.61 $\bar{X} = Ø.57$ Br (.61) I (.54, .54, .62)

1.43 SO_3 (.69, .57, .57)

3.42 HN_3 (3.34)

~~3.66~~
~~2.61 Na (2.11)~~ Exclude

2.32 Mg (2.36)

~~3.02 Li (3.04) K (2.93)~~ Exclude

3.26 HN_3 (3.34)

1.83 H_2O_2 (1.81) (1.78) Maybe not so unclear

We also have a 1.93 unexplained

$\bar{X} = 1.87$ N_2, NH_3OH (1.83)

Al Cl (1.80)

Remains unclear

You remain led to strongly Consider

* HN_3, H_2O_2, Halogens (Cl, F, Br, I) Mg (or electrolytes)

The analysis of these different reaction sets of 12-20 ions each a presents a consistent pattern where the following components are to be strongly investigated:

1. HN_3 - nitrogen mustards
2. Halogens (eg Cl, Fl, Br, I)
3. H_2O_2 - peroxide
4. Electrolytes - eg Mg
5. Fe

additional Considerations:
SO_3, SO_4, Al, Ca

Iron and Iodine continue to show target interest.

Apr 18 2022

The data files are being analyzed chronologically from most recent to oldest. The cumulative scan is being reviewed. You have completed two files thus far. File saving is used as the date criteria, not the name.

File Name:

1. R+L Apr 13 2022 1745
2. L&R Apr 14 2022 1030

Next file is:

R-L Apr 13 2022 0245 +1.0 to −1.0V

y° DC y' DC

+Ø.70 (Fe^{+3}, Fe^{+2}) Ø.77 Fe^{+2}(Ø.74) Br(Ø.77) SO_4(.75) (1) Ø.73

+Ø.52, +Ø.52 I_2(.54, .54) Br(.61) SO_3(.66)

Previous avg of Ø.57 : (.57+.52)/2 = Ø.545

+Ø.91

+Ø.98

We see that y' for the DC component generally seems to be the most useful graph info.

Iron and Iodine reoccur as prime targets here other considerations remain as Bromine and sulfates, sulfites to a lesser degree. Note on aromatic ring remains

Next file "

Our next file of interest is:

L-R Apr 13 2022 0225 -1.0V to 1.0V

4' DC

+Ø.18 (SO_4, Ø.17) we had a -Ø.12 earlier

-.77 (Fe^{+3}, Fe^{+2}, .77) Fe^{+2} (.74)

-.91 (Notice previous +Ø.91)

+.55 Previous avg at (.545+.55)/2=.55 I (.54)

Iron and Iodine once again of target here.
Sulfate interest repeats

Ø.91?? NO_3 is (Ø.96) NO_3^-, NO g
This is now added to target list.

CDB Protein (in Blood) analysis; 4 Files Analyzed
The target list is now: Cumulative Scans
n=12-20

1. Nitrogen mustards HN_3
2. Halogens (Cl, Fl, Br, I) emphasis upon Iodine
3. H_2O_2 peroxide
4. Iron
5. Electrolytes (eg Mg emphasis)
6. Sulfates
7. Nitrate NO_3^- (Nitrate is NO_2^-)

* This is the target list

The next file is
L-R Apr 12 2022 2250 −2.0V to +2.0V

4' DC

+0.50 This is a definite peak $(.55(3) + .50(1) + .52(1))/5 = 0.53$
+.52 (I is 0.54)
−1.72 (H_2O_2 is 1.78)

Peroxide and Iodine are once again prime targets
The list above remains intact.

Our last file available

L-R Apr 12 2022 1250
It looks to be a substantial file w/ n=20 scans
It runs from -3.5V to 0

4° DC	4° DC
-3.37 HN_3 (3.34)	-3.48 HN_3 (3.34)
-1.50 (Cl, -1.46)	-1.76 (Fe^{+2}, +3, 0.77) Fe+2 (1.74)
	-2.36 Mg (2.36)

There additional confirmation take place here

The target list remains intact and is further confirmed.

~100 scans w/ ACV are involved in the analysis. The target list contains highly probable components (primarily of an inorganic analysis) of the CDB generated foreign protein within the blood sample.

All blood samples that demonstrate the unusual coagulation behavior (ie, to date all samples that have been analyzed) are anticipated to contain the protein and microorganism, ie CDB.

It is strongly postulated that the COB-protein complex is responsible for the extensive unusual coagulation that is taking place within blood samples.

All blood samples thus far are of unvaccinated individuals and the vaccine(s) virus has not yet been introduced into the analysis. Until samples from vaccinated individuals come into the picture and are available,

The "vaccine virus" is not required to demonstrate that an issue of global scope is already both resident and sufficient to produce lethality.

Apr 19 2022

I am going to see if I can collect a NIR spectrum on the concentrated-purified CDB-protein complex.

There will be additional useful identifying information beyond the extensive electrochemical AC Voltammetry profiles that have been collected.

My greatest difficulty might be the amount of sample remaining. I am now at about about 0.25 ml of remaining liquid sample.

My first sample attempt will be to use 50 ul of solution and to allow it to dry. We will see if sufficient signal strength exists.

The work now attempts to focus on the organic aspects of the sample, in contrast to the inorganic focus of electrochemical study.

It is worth noting that the solution (alkali w/ NaOH, KOH) is of greenish tint wherein the CDB-protein complex-isolate is now dissolved.

The fact that the isolated and purified solution has green as one reason for the simple explanation that it is not blood.

Green tints often come from iron presence (Fe+2)

We do indeed have a sufficient signal. There may be H_2O left in the sample since we dried, so I will monitor for. CH_2 2+1+1+1+1=6 ✓

ArOH: 1+1.5+2=4.5 ✓ CH_3: 3+1+3+1+2+3+3+2=18 ✓

CONHR: 2 ✓ Peak Data ROH: 3+1+3=7 ✓

$CONH_2$ 3 ArCH=1 CH_1: 1+1=2 RNH_2=1

	λ	Abs	
	933nm peak	.096	CH_2(2) CH(3) ROH(3) H_2O(2)
	1123 ascending inflection	Ø.09	CH_3(1)
	1169 peak	Ø.107	CH_3(3) CH_2(1) CH(1)
	1283 ascending inflection	0.102	CH(1)
	1352 ascending disturbance	.142	CH_3(1)
	1366 ascending inflection	.153	CH_3(2) CH_2(1) ArOH(1)
	1375 ascending inflection	.168	CH_2(1) ArOH(1.5)
diminishing w/time	1435 peak (strong)	.362	H_2O(3) ROH(2) ArOH(1) $CONH_2$(1) supercede
	1644 descending inflection	Ø.186	CH_3(2) ArCH(2) supercede

As the sample dries, the H_2O peak is lowering & flattening out. It now consists of SUPERCEDES:

	1420 peak	.241	CH_2(3), ArOH(2) H_2O(1) ROH(1)
H_2O minimal now	1451 descending inflection	.245	CH(3) CONHR(2) $CONH_2$(3) ROH(3) H_2O(1)
	1650 descending inflection	.118	ArCH(1) CH_3(2) CH_2(1)

As the sample continues to dry further we have an additional:

λ		Abs	
1564	descending inflection.	.071	

There is no reference signal here. However close inspection shows a very subtle peak inflection @ 1539

1539	subtle peak	.085	RNH_2 (1)

Our closest signal, for which there also lately is no other competition, is RNH_2 (~1400 - 1520, 1525).

Though we have the following functional group candidates ranked by score. Regardless of score, each functional group is likely to exhibit w/in the complex.

Group	Score	Rank	
CH_3	18	1	Higher scores, or ranks, are expected to increase the probability of existence.
ROH	7	2	
CH_2	6	3	
ArOH	4.5	4	
$CONH_2$	3	5	
CONHR	2	6	
CH	2	7	
ArCH	1	8	
RNH_2	1	9	

The presence of ROH, ArOH & CONHR and $CONH_2$ are regarded as significant here.

The would explain the ~~level of solubility~~ a level of solubility in the blood, as well as make the case for aromatic substitution chemistry w/ the halogen existence, as well as being of a protein nature.

It can be said that the NIR analysis has provided a meaningful contribution to the state of knowledge on the CDB-complex nature.

Summary of NIR interpretation:

1. Soluble (to some degree) (pH dependent)
2. Aromatics likely
3. Protein Existence

APR 19 2022
CDB-Protein
Complex
NIR Sample
50 ul

A feasible reaction to explain the green tint is that an iron salt, such as iron(II) sulfate + KOH → $Fe(OH)_2$ iron(II) hydroxide + potassium sulfate.

When exposed to some oxygen (as in water) iron hydroxide has a green tint

There is another method of demonstrating the existence of iron in the COB-protein complex in addition to AC Voltammetry

Insoluble @ pH 7.0

Formula is $FeO(OH)$

Color varies depends upon pH and level of exposure to oxygen.

We have
1. In water
2. for alkali (pH >> 7)

Conclusion: green tint.

We also have the NMR spectrum by itself regardless of the constituents involved. Has the unique identifying characteristics.

Apr 22 2022

I have an idea shaping up. I am going to subject the protein to an enzyme mix and see if it has any effect upon the protein concentration as determined by VIS spectrometry.

Also, there are some variations in use of the VIS spectrometer such as fluorescence, kinetics, etc that could also be worthy of investigation. It is actually rather capable software.

The idea - the subjection of the isolated protein to enzymes as an follows.

1. Use the protein red dye reagent as the reference.

2. Prepare a solution of the reagent w/ ~ 100 uL of COB protein concentrate added that should produce the Cola shift and so consequent protein detection spectrum.

3. Apply a microscope quantity e.g. < .01 gm of enzyme complex to the protein dissolved sample.

4. Look for any reduction in the magnitude of peak absorbance in the protein solution w.r.t. time.

Our reagent (protein) may and appears to be turning more orange in color with the passage of time.

This is not necessarily a problem but lets test the titration w/ a milk protein control first.

One thing we see is that our portable powered stirrers that I bought for an altered coffee whip work perfectly!

I will start out w/ 20 ul of the protein (milk of moderate concentration) added. It should be rather weak.

I have very detectable absorbance occurring @ 5 [illegible] different locations with my eyeball and they do increase gradually in magnitude w/ time as desired.

There are very different signals:

		Abs
384 nm (UV!)	peak	0.112
426 nm	peak	.051
exclude ~~463 nm~~	~~shoulder~~	~~.035~~
905 nm	peak (or shoulder)	.046
939 nm	peak	.096

Now we select and read the reference. (~15-20 min elapse now)

Absorbance in milk protein control @:

λ	Abs
383nm	.066
419 nm	.042
435	.039
~~462~~ exclude	.029
907	.024
937	.047

the ratios could end up key (new + additional peak)

important to analyte. 419/462 = 1.45

A high level of uniqueness and repeatability here.

It certainly does appear to be far more advantageous to use the reagent as the reference vs the chain of H_2O reference vs Reagent sample vs Protein sample.

There is now a highly discernible spectrum that should easily demonstrate change.

Let's look @ the 907 & 937 NIR peak

907 CH_2(3) CH_3(2) ROH (2)

937 CH(3) CH_2(2) ROH(3) ArOH(1)

Now what is the dominant structure of milk protein?

Casein is 80%

Structure:
$C_{81}H_{125}N_{22}O_{39}P$

Well it definitely has ArOH & ROH & CH_2 & CH_3

so there is an excellent match.

~~In addition an absorbance peaks in the UV and~~

Ok, what I have done now, as an additional stage in the control process is add 30ul of a moderately concentrated enzyme mix to the milk-reagent solution.

Spectrometric monitory is now going to be required and just previous to please we know that the Cal actually require a process involving several days.

Now what is also realized here is that using the reagent dye combined with the milk protein is probably our best reference solution to use.

The mean that Change will be more detectable in that fashion.

We do, however, also already notice some change in the spectrum taby place.

There seems to be increased absorbance in the green-yellow orange portion of the spectrum. It is of low magnitude but it is nevertheless present.

420 .056 420/466 ratio = 1.39 vs 1.45 prior
~~exclude 466 .036~~ Some change there but not sure if it is significant or not.

383 .162 .162/.055 = 2.94
419 .055

383 .066 383/419 prior is 1.57
419 .042.

The, however, appear to be a very significant difference. The indicate that the lysozyme have indeed having a significant effect upon the casein protein-lipid micelle

.935 .094 .094/.060 = 1.57 w/ enzyme
.906 .060

.937 .047 .047/.024 = 1.96 prior
.907 .024 w/o enzyme.

The is also potentially significant.

937 nm is most likely to be attributed to the presence of ArOH.

This indicates furthermore that the conjugated bond of ArOH may have well been destabilized or broken down.

In addition, the increase of absorbance @ 303 also would indeed indicate a decrease in the number of conjugated bonds

further indicating a deterioration of the protein structure.

This is an ideal and somewhat fortunate confirmation (and hopefully) outcome associated with the previous hypothesis, and that is that enzyme may be very beneficial in helping to break down the fava protein in the blood

We therefore see, w/ a lapse of time of ~ 1 hr exposure of milk to enzyme, three different signals:

1. An increase in the 303 nm UV absorbance
2. A broad mild increase in absorbance from ~500 - 650 nm
3. A suspected decrease in the ArOH component by exclusion @ ~935 nm.

Now let's change our reference solution & see if we can make this evident (more so)

Reference now to be: Protein Reagent + Milk + ~~Enzyme~~ 20 ul

Then look @ change from the reference w/ the addition of enzyme.

Now we realize that we also need to let the reagent reaction w/ milk develop to a level of stability before we add enzyme. I will estimate that this process will take 15-30 min

Ok, our protein-reagent reaction certainly seem to be reasonably stable here - 30 min between aliquots

λ	Abs	w/Enzyme after an additional 30 min
383	.114	.142
~420 / 440	~.064 / .061	.062 / .062
exclude 464	.045	.041
493	.021	.029
909	.050	.065
942	.109	.117

Now we add Enzyme. 30 ul

Actually I messed it, forgot to adapt the new reference, but I can still proceed & then repeat.

.142/.062 = 2.29 vs .114/.061 = 1.87 = +22%
.117/.065 = 1.80 vs 1.95 = -8%

We therefore have essentially the same results as before. An increase in the 383 absorption and a decrease in the magnitude ~~rating~~ ArOH ~~—to~~.

Now let repeat once again ~~what~~ using the modified reference described, 20 ml milk within cuvette volume of reagent.

We do notice that the tint of the reagent dye has shifted from a more reddish tint to a more orange red tint, but it seems in no way to change its effectiveness as a sensitive protein detection reagent. There is a marked shift in the spectrum when protein has been added to the reagent as has been recorded during the session. It may actually be even more sensitive.

The reagent-protein reference to be adopted is now accepted as ~~stable~~ stable over a period of 30 min. This will be the newly adopted reference, ie reagent + milk, and enzyme will be added to that reference and any spectral change recorded.

(~20 min seems to actually be sufficient)

Ok, this method works superbly. The spectral sensitivity to change is dramatically increased w/ the proper selection/identification/establishment of the reference solution.

In this case our reference is Protein Reagent + Protein.

It is found that when enzyme are added, a very significant spectral signature of enzyme activity can be captured.

It shows they to be exactly previously identified along with other additional and considerable activity. We have definite identifiable peaks at:

After 30 min Activity Desired.

Peaks nm	Abs
383 sharp peak	
411 local peak	
exclude ~~466 local peak~~	
514 broad peak (500-600 nm) (also weak)	
800 - 910 nm (broad NIR absorption band	
908 local peak	

The net effect of this is that it shows that the enzyme complex has very significant structural impact upon the Chemistry of Milk protein, namely Casein. And the structural impact is another name for digestive breakdown.

Iron bound proteins may well not show the level of activity that we will see.

3 hours Later:

There is now a very important Control observation to make. The enzymes have made an even more dramatic impact upon the spectrum over a three hour period vs the 30 min period. All activity is important here, but the latter spectrum show how important the enzyme activity can become.

It can be regarded as a ~~slightly~~ highly modified spectrum now.
Here is the identifying information:

	λ	Notes	Abs
	379	Noticeable shift to left At limit of range of instrument	0.116
	403	Also Significant shift ~~to~~ left local peak	.042
exclude	~~467~~	~~Now a left shoulder~~ on a broad major peak	.017
	505	A new left shoulder on a broad major peak	.029
	522	A new left shoulder on a broad major peak	.039

		Abs
539	Now a major central peak	.042
800	Now a distinct local NIR peak	.019
863.	Now also a local NIR peak	.018

The 908 local NIR peak IS NO MORE!
Absorption is in a process of sharp decline to ~.001 @ 908 now

The spectrum shown how dramatic the influence of enzyme on a certain protein (in this case Casein, can be ~~about~~ over time.

The has now become an important analytical tool, ie ~~et~~ enzyme activity via use of a sensitive protein reagent test combined w/ VIS spectrometry.

A similar endeavor may await us with NIR if suitable sample material can be developed, ie non aqueous. But VIS spectrometry does show it the case.

I have photos of the spectra @ 30 min & 210 min recorded graphically, the result are rather impressive.

I will undoubtedly be looking @ the CDB protein isolated from blood in the fashion. It must be acknowledged ahead of time, however, that that sample has been subjected out of necessity to NaOH & KOH for solubility purposes. The results however are still of keen interest as that is primary a pH issue.

We must equally acknowledge that it would be difficult to regard any blood as "PURE" now and so even so-called Control studies involving purported "PURE" (or expected pure) HEMOGLOBIN must be regarded as intrinsically suspect & fallible.

It will nevertheless be of value and interest to conduct similar work upon blood and "hemoglobin". PH Characteristics here of blood vs isolated CDB-protein complex, however, may end up being of much value.

I am now establishing the reference solution for the Protein Reagent - ~~CB~~ CDB protein complex. I am ~~a~~ almost out of my CDB-Protein Concentrate solution. It should take 1-2 days of solid work to produce ~ 1 ml of the CDB-protein complex so it rather precious @ this point.

My reference solution is any 50 ul of the protein concentrate in ~3 ml of protein reagent and I intend to let the spectrum settle in over ~30 min as before.

The reference spectrum developing does, on first glance, look very similar to that produced by the milk protein (Casein). We have the following spectrum developing

λ	Notes	Abs	
383 ✓	Strong peak	.134	We see that
420 ✓	peak	.071	our results
436 ✓	parallel peak to 420	.069	here are
exclude ~~465 ✓~~	~~Rt Shoulder weak~~	.052	essentially identical
493 ✓	Rt Shoulder, weak.	.024	to that of
906 ✓	Left Shoulder moderate	.060	milk protein.
939 ✓	peak	.099	A favorable result.

Ok, the reference is in place. Now let's use it. 30 ul enzyme.

Peak absorption of protein is @ 280 nm.
We can't go that low. DNA is @ 260 nm
260/280 Ratio: "It is relatively easy to see if you have DNA in your protein prep, but it is harder to tell if you have DNA in your protein prep" No DNA work in this lab alone, just curious about UV absorption

"Heptane" apparently absorbs @ 383 nm.
Heptane is a straight chain alkane $H_3C_5CH_3$ or C_7H_{16}
No double bonds!

I have generally verified the 383 nm absorbance peak by heptane with a UV spectrum. It shows double bonds are likely required to show UV absorbance - keep that in mind.

Spectrum started @ ~ 30 min.

It is not as well defined as the casein trial was. Our peak structure has:

λ	Notes	Abs
383	Strong peak here as before	.042
406 nm	left local peak of noisy spectrum section from 400 - 625 nm	.020
909 nm	Left shoulder peak moderate	.008
944	peak	.015

40 min into the spectrum with a reset of the instrument, we are now seeing similar behavior developing as with the milk protein.

Once again we do see a major lobe developing @ 532 nm

It is indeed likely they appear to duplicate the behavior they exhibited on the milk solution.

It is however, only mutually developing as compared to the 3'2 in spectrum.

1½ Hours into the spectrum development:
(2 hrs total now)

Watching the enzyme influenced spectrum develop is quite interesting. It is a very gradual process and in general the same pattern of absorbance is developing w/ the COB-protein Complex as it did with Casein (milk).

It does seem to be more subtle, slower, and less pronounced. We do, nevertheless, have—

1. Noticeable increased UV absorption in the 383 area.
2. We do have peaks forming @ 406 & 532 nm but they are, so far, less pronounced than they were with Casein! The 532 peak does, however, continue to gradually broaden and increase in magnitude where it remains to assume a dominant presence.

3. In the NIR range there is a reduction in the 934/907 ratio commensurate w/ the milk but the substitution w/ the 800-860 nm is not @ all pronounced. Also the somewhat dramatic loss of the 934/907 peaks is not taking place thus far.

We do have very common behavior and general, it is quite not so obvious or pronounced then Gar as it became w/ the Casein

nevertheless, the hypothesis of enzyme may effective in the reduction of structure of the CDB-protein in the blood

* LOOKS TO BE BOTH FAVORABLE & VERY COMPELLING.

—

One of my future trials will, in the pursuit of completeness, replace the same behavior with blood (even though it is preserved now to contain the foreign protein as well).

We should also realize that our CDB-protein concentration is likely CONSIDERABLY LOWER than it is for the Casein sample, and this could easily explain the lower magnitude of response but maintaining the same general character of response.

—

Two hours into the spectrum (2½ hours total) we see that the 934/907 ratio continues to equalize and is now approaching unity.

The 532 band has peak continues to decrease steadily in importance

and the 383 peak continues to gradually increase in magnitude. The behavior is showing increasing similarity to the behaving enzyme upon milk.

Here is a question: How do you know that the enzymes are work primarily on the protein constituent vs the reagent?

You know w/ an additional control imposed:

① Reagent + Enzymes — no protein added.
You need to see that spectrum behavior over 3 hrs also.

② 2nd Comparison will be with Reagent + Blood + Enzymes

Two major control projects ahead.

We therefore know enough now to conclude essentially identical spectral behavior between melt & the CDS Complex under the influence of enzymes.

Let's look into the Reagent + Enzyme question.

So now for another enlightening viewpoint.

Look @ the Reagent reference itself over time BY ITSELF.
Also time it for the 30 min.

Here is what we see. The reagent by itself, even when adopted as the original reference solution. Does show the following peak endogenous

383nm	.07 (3min in)
420nm	.03
907	.022
936	.044

Notice from almost the onset that we have a 936/907 ratio of 2 to 1.

~~Now the question~~ Now a question will be does that ratio decrease when enzyme are added, let alone if protein is added?

The 382/420 ratio is on the order of .081/.037 = 2.19 so 382 does show prominence & also evenly stay w/in the reagent control process.

The ratio is also consistent w/ our very first onset of reference work today, which has a with Ruby dust + protein (i.e. legume had any time to react.) (i.e. 384/426) = .112/.51 ≈ 2.1

Then after 15 min we see the reference and we see that the ratio declined 383/419 = .066/.042 = 1.57 so it decreases as the protein (casein) reacted with the dye.

1. As a couple of observations. The 532 broad dominant peak ~~fluctuation~~ becomes is definitely not happening.

2. A decrease in the 382/420 ratio is definitely NOT happening.

3. A decrease in the 936/907 ratio is definitely NOT happening.

Even after ~20 min into the reference question.

The a they're extremely important control experiments. These ratios are SO, to be very important in determining when exactly Lyn kinase activity and what it is they caused by.

I think I do not want to reset the reference right now and let the process unfold. There is logically no need to reset @ this point since nothing more has been added to the reagent. At the end, however, show interesting behavior of the reagent over time and we see that the is critical to understand.

Now let's look @ when we add enzyme to the reagent alone - NO PROTEIN, Add 30 ml enzyme

We have now added the enzyme.
Let the unfold for a few minutes.

We see the emergence of the mono shoulder peak @ 465 or nm. This peak is now to be disregarded and excluded for analysis

At this point there is no additional significant impact @ 5 min time.

1. No ratio reduction @ 382/420
2. No ratio reduction @ 936/907
3. No Broad peak formation @ ~532nm.

Again, valuable control info here.

Important Signals & Markers of Reagent + Protein + Enzymes Reaction

* There are now telling us that the major signals of enzymatic protein reaction are

1. An equalization or diminishment of the 936/907 ratio.

* 2. The development of the broad (and potentially shoulder) band peak centered @ ~532 nm. The peak is completely absent the gel without the existence of protein, even with Reagent + Enzyme

* 3. Possible peak formation ~ 500 to watch for

* 4. Change in the NIR region also appears to be very significant & monitor, excluding the 900 - 960 region in addition to the equalization or diminishment of the 936/907 ratio.

There is a very important observation of the enzymatic influence.

The conclusion thus far is that the application of enzyme to the CDB-protein complex has shown that is perfectly in accord with what is observed in the reacting cascade.

This is interpreted as a structural breakdown occurring w/ host proteins.

The suggests are very very important because
likely to ensure to cause a reduction
in the CBB - protein complex, in the blood on
the use of enzymes on a regular sustained basis.
We must, however, keep an accuracy that these
too strongly absent the impairing the
reaction of blood. ↑ which I already do.

Now we know that blood has the large
protein already in it so the study / needle
the water already. You would then see
the spectral behavior already clarified
without much doubt.

It seems like what is necessary then to repeat
this study to include hemoglobin so that
you must acquire some "purified" hemoglobin
then bring you back to your earlier electrochemical
& centrifugation separation practices. Obviously
rather complex and involved.

Incidentally the enzymes under use here are
"NOW" brand - "Super Enzymes"
Contains bromelain, ox bile, pancreatin, & papain.

* The theses are that digestive enzymes
* can be shown to break down the structural
* nature of the CBB - Protein Complex that now is
known to commonly exist w/in blood.

Apr 23 2022

I am seeking to replenish my protein concentrate via use of SCV. It may be that the scan set to 0 -3.5 V to 0 is more productive than from -3.5 to +3.5. Be aware of a potential cancellation factor using both pos & neg voltage values.

I am now using -3.5 to 0.V and I do see initial fast response.

I think that one they grow as the blood concentration is higher, I think that it requires more scans to initiate & most certainly complete the process. I am in 23 scans of 2 min apiece then is no real separation of the blood solution yet and only preliminary foam-precipitation developed.

I think a question is whether or not a steady voltage can be applied vs a scan.

It looks to me like straight potentiometry might be able to apply heat to try out.

What you do is apply a current. For us at bottom left -2mA would be a representative value.

I am indeed going to try this. It would certainly make data collection much easier if it continues to produce the precipitate.

I am actually using the Chronopotentiometry mode. I have it set to use

$I = -3\ mA$
$t_{interval} = 0.1\ sec$ (10 pulses per sec)
$t_{run} = \cancel{600\ s\ (10\ min)}$ 2000 sec 32 min

$n = 6000$ data points. Let's see how we do.

The current of -3 mA is delivery about $-2.5\ V$

$E = \frac{I}{R}$ $\quad R = \frac{E}{I}$ $\quad \frac{-2.5\ V}{-3E\text{-}3\ A} \approx 830\ \Omega$

$P = I \cdot V = 3E\text{-}3\ A \cdot 2.5\ V = .0075\ W = 7.5\ mW$

This is what should generally be happening w/in the sample. Power applied on order of 10 mW & $\Omega \approx 1k$

This will certainly make life a lot easier if it works to have 30 min scans instead of 2 min scans.

It does look like there is quite a lot of precip that has collected on the working electrode.

You can see gas bubbles produced by the electrode so you know the sample is active.

I can see that the foam prep is now gradually and steadily increasing

This is perfect.

OK, the question arises what does "foam" mean, chemically speaking?

The search results are already interesting.

"Natural suds result from the decomposition of plants and animals. This is commonly referred to as "dissolved organic Carbon" which causes air bubbles, reduces the surface tension of the water, and forms mounds of dense light colored bubbles. The DOC is naturally occurring foam provides energy for the stream's complex aquatic system..."

"Foam produced from natural causes is identified by its off white color and its "fishy" or "earthy" odor."

The second source is even more direct:

Azmea KF7GC Tom

It is about "foamy urine" from the Mayo Clinic

If you have persistently foamy urine, this can be a sign of protein in your urine.

Increased amounts of protein in urine could mean you have a serious kidney problem"

Another:
"Biochemists know that a protein solution beginning to foam is always bad news. It means the protein is losing its structure" "Very few proteins are naturally meant to foam"

And so.... I am breaking down the COB-protein complex that is now found to be within blood.

The idea of a constant voltage (DC) was a good one, sample collection will be much easier and more efficient now).

You might be able to increase the current flow

I am now using & subject the sample to -10mA. The is producing a potential of ~-4V

$I = \frac{E}{R}$ $R = \frac{E}{I} = \frac{-4V}{-10E\text{-}3A} \cong 400\Omega$ $P = 10\text{-}3A \cdot 4V = 40mW$

The rate of foam (ie, protein) production is indeed much quicker under these circumstances. A current flow of 10 mA is most definitely an improvement over 3 mA.

I am now seeing greater control over the production and isolation of the COB-protein complex.

We have very good foam production, separation & collection.

Recall that we used 10 mA constant in these extended runs of 30 min apiece (3-4 runs)

Now what we noticed is that we have the formation w/in the main vial of what appear to me to be the equivalent of a "blood clot", ie we have layer & independently the material on the bottom of the tube.

We therefore have quite a few separations taking place w/ the separation of blood.

With an foam layer subjected to centrifugation we once again have our 3 layers. Let recall how the extraction process developed.

From the Apr 06 notes, it appears that we used the central layer of transparent solution and subjected it again to electolysis, for the past a precipitate was formed.

Well, that certainly was an unfortunate event.

The glass sample test tube that was in the centrifuge busted through the bottom and I lost my entire sample, just as the separation was ending.

I have lost the entire days work.

But I have established some new methods that will increase the rate of protein collection in the future.

But it is indeed a real shame to have lost it.

I suspect I will need to keep the centrifuge ≤ 3000 RPM in the future but the fact is that should not have happened. If it happens again I will probably need to shift over to the plastic test tubes which I do not like.

You must start the process all over again that I was a good sample I had prepared.

VOID Discarded

You do have a sample of the coagulated appearing blood in the original electrochemical vial which can be worthy of examination.

VOID Discarded

You have been able to recover one last small foam sample that you can continue to process. It will be absolutely monumental relative to what you have had accomplished today.

I think on century one in future you will need to keep it in a range of $\leq$ 2800 RPM and just let it run longer.

I am going to see if I can accelerate the process even if little. I have created a new blood sample.

I am now subjecting the blood sample to −20 mA Chronopotentiometry instead of −10 mA and instead of 3 mA. The −20 mA is producing power again a max voltage of about −4V. $I = \frac{E}{R}$ $R = \frac{E}{I} = \frac{-4V}{-20mA} =$ 200 Ω

$P = IV = 20E\text{-}3mA \cdot 4V = 80mW$.

I also notice now that foam precip is being generated at both Red & black electrodes.

Repeat collection is going well. You have probably increased the rate of production by ten fold. I will estimate that it will now probably take 2-3 hrs to process the sample collection now.

Ok, good news. In about an hour of time I have collected enough foam - slurp - protein to likely obtain a washed extract.

I am back in the centrifuge @ ~3000 RPM. It is running very smooth and it is a surprise that the previous test tube suddenly broke & collapsed from the bottom.

I am estimating that I have collected as much as 10 times as much material as the earlier run. I have separated the lower solution layer of the "1st flotation centrifugation".

I believe the anticipation is that we will see a precipitate form. Let's check on that.

The work was described on Apr 07 2022. This is what I am now attempting to reproduce. I am now using Chronopotentiometry vs Ag/AgCl now and I am applying -10mA. The voltage output is approx -4V.

I do indeed see a brownish sediment forming in the vial and that was an encouraging sign. I will monitor the vial and see if replication occurs as the Apr 07 work.

For clarity sake what we are seeing is actually 3 layers forming again, that is not the case as Gen 1 started.

1. We do see additional foam like product on a top layer. It appears to be much more pure than w/ Gen 1 attempt
2. We do see a brownish layer in the middle comprising the bulk of the vial.
3. We do see a blood colored layer settling to the bottom of the vial.

Remember that the sample may contain considerably more mass of sample than our Apr 07 runs.

The three layer separation under electrolysis is taking place even though the sample initially exists as a uniformly colored solution (red tinted) w/ no solids visible in it as it was extracted from the center of the 1st-generation centrifugation.

— Post processing:

All signs are now that we have actually isolated two separate proteins (foreign) from the blood.

#1 One of them is water soluble.

#2 The second is of solid form, but can be made water soluble with the addition of a small amount of KOH – NaOH that alters the pH.

I have considerable more volume of isolation now available. There is due to significantly modified method that now use Chrono potentiometry vs ACV Voltammetry. Chronopotentiometry is well suited to isolation & extraction when combined w/ centrifugation. ACV is well suited to inorganic analytes.

#1 is of a pinkish tinge. It is clear and transparent. If shaken it produces suds/foam. I now have a full 10 ml tube. Water soluble.

#2 is solid, of tan/brown color as isolated previously. It is isolated by Centrifugation. It is dissolved easily in a small quantity of water, eg 1 ml, with added KOH – NaOH in this case 30 ul. It will turn a greenish color upon dissolving and has been deduced as containing iron(II) hydroxide.

These two separate proteins have been isolated, in rather pure form, by a two stage alternative process of chronopotentiometry "CP", separation by phase (by solid, liquid) and centrifugation.

The sequence is thereafter:

1. Dissolve a couple of drops of blood in ~ 4 ml H_2O

2. Apply CP in total for ~ 2 hrs.
   Current level applied is ~ 20 mA
   Voltage at pt will be on the order of 3-4 V.

3. Foam precip will result on a top layer
   Solids (of bright red color) will settle on bottom
   A reddish tone liquid will form the bulk of
   the sample that remains; it will become
   transparent.

4. The target material of interest here is the foam precip. This is a distinct separation from the blood. This is what is collected and dried & placed into a small volume of water, eg 4-5 ml. The foam nature alone indicates that it be a separate protein from the blood, which will eventually settle to the bottom of the tube.

4.5. The foam precip that is placed into a small volume of material is then centrifuged.

3 layers will again form.
1. Lighter materials that have been clearly to demonstrate the existence of advanced CDB filaments.
2. A transparent liquid, of a peculiar nature that appear to be homogeneous, & transparent.
3. A small amount of solids that will centrifuge to the bottom.

6. The target material now become the homogeneous transparent solution of pink-tannish color. The is extracted from the center of the test tube in the most pure form possible.

1. Now we apply CP again, for an additional period of ~2-3 hrs. The was done @ -10mA vs the first elution separation @ -20mA. It is very clear that a second process of purification and separation is taking place w/ the second elution CP.

B. There layer will again form, but there is an important difference:

1. A drastically smaller foam precip layer will form @ the top again.

2. A precipitate will form @ the bottom of the tube. This will be of a tan or brownish color; this is a unique phase of the process and is of a distinct visual nature as opposed to original red (hemoglobin) specimen.

3. The solution that remains will become almost completely clear w/ little to no color @ the end of the second generation CP.

9. This collection of the three layers, which is combination and total is distinct from the first generation CP is again Centrifuged.

10. Two layers will now form

1 Distinct clear transparent solution that when shaken will produce suds / foam

2. Solid material at the bottom that are of a tan / brown color. (To be dissolved in lye to produce a green water soluble liquid)

The places us back to the point recorded in the "Post Processing" previous section.

I now have a very sufficient volume of separated and purified materials for further (and secondary testing).

What is different this time is that I also have a fair volume (~10 ml) of what is most likely an additional water soluble protein in addition to the ~1.5 ml of solid protein of tan/brown color that had been dissolved in lye to produce the green color.

I am now establishing an additional control on what is being called an additional (potential) "water soluble" protein. Because the solution has a greenish color, it will be compared w/ highly dilute pre-treat blood study. The only visual discernible difference between the two tubes is that the potential 2nd protein of proposed water soluble form has a slight whitish tinge to it in comparison to blood study and it also produces some milky when shaken (low volume). Concentration of any additional dissolved protein can easily be a factor here.

It is plausible here that we have dilute blood w/ a secondary protein of low volume contained w/in blood. I will see what I can do w/ it.

The solid protein is of great abundance, listed as tan/brown in color, reacts w/ lye, and has already been extensively analyzed.

As an additional control, fresh blood in H_2O does turn green with KOH - NaOH is added. Keep this in mind as we continue to separate & identify.

Let's think about how sequences and characteristics is to be established here.

1. NIR of blood control vs 2nd Gen CP separation

2. Electrochemical profile of blood control vs. 2nd Gen CP separation

3. The microscopic record of foreign materials in the blood.

4. Clear NIR separation of blood vs Foam

As you control work, however, you must also consider the possibility of "decomposition" of hemoglobin itself by electrolysis, in addition to the (a reverse) act of separation of a protein from blood. (foam)

* There is a very important aspect of serology testing here.

We do know, however, that positive identification of CDB morphology, ie, advanced filament structure and extensive CDB themselves have been repeatedly identified as a direct result of the electrochemical processes that have been applied to the blood.

Equally important *

Microscope sights need more use upfront here.

Apr 26 2022

Status: We know that we have some very effective electrochemical separation processes in place now. Our arsenal includes

1. Chronopotentiometry (CP) effective for separations
2. ACV (AC Voltammetry) effective for inorganic identification
3. NIR - effective for establishing uniqueness in identification, and for fundamental 1st level organic analysis.
3. VIS spectrometry (& minimal UV & NIR) - effective for protein detection
effective for identification analysis

We have also radically increased the pace of separation electrochemically by switching over to the simpler process of CP, which demands every bit as effective.

An iterative process of CP is very effective at increasing the purity of separation

Our current situation is that we have a series of separations that appear to be fairly pure. The weakness is that I do not have the upfront VISUAL MICROSCOPIC examination of the various separations in place. The known exists as an obvious need since the effort of further identification and analysis by instrumental methods such as ACV & NIR are much more demanding in terms and effort expended.

SO!

We need to LOOK (once again, the obvious, which we so often NOT DONE) at our staged sample separations as we proceed before coupling additional instrumental analysis.

The was done on the first and major discovery separation w/ the USE of ACV Beasley & some very profound results and recorded observations (under the microscope (eg abundant advanced filament presence).

It was not done on the second run (using CP) and it shows up as a deficiency.

Let's see what we have again along each important stage along the way.

One of my questions that has arisen along the way is the degree of separation from hemoglobin protein that can be established.

We know that we have important differences such as:

1. The presence of advanced filament growth w/in our separations
2. The formation of a brown / tan precipitate vs that of a blood color
3. Which also does test positive for protein

However, some points of confusion + uncertainty remain:

1. We know that we have a foam - precip product. We know that the denaturation of proteins are very much the likely cause of our foam production

2. But how do we know that hemoglobin itself and the potential deterioration of hemoglobin itself is not a factor here?

3. Both goat blood and our "tan precipitate" turn green when exposed to light. This indicates Fe(+2) hydroxide. But are they the same or is there any hemoglobin overlap here? How do we know?
   - VIS spectra way?
   - NIR way?
   - ACV way?

4. The color of the base liquid of even the 2nd filtration separation does have a yellowish tone to it. I have run a control w/ highly diluted goat blood and at least a visual level I can produce a semblance test. The difference in our separation contains a reddish yellow aspect to it and is also a lot whitish in color.
   Once again, further characterization analyses is required

So, we'll now have a little handle on what levels of distinction exist

WE LOOK @ each important stage along the way UNDER THE MICROSCOPE.

Of course, another major difficulty lies in that * the existence of "PURE BLOOD"

* Can no longer be shown to exist.

* This makes life in the lab much much more difficult.

Blood Composition info

Hematocrit is the % of red cells in your blood.
Note Male hemocrit levels 44-50%
female hemocrit level 36-48%

Hemoglobin makes up about 96% of the red blood cells dry content by weight, and about 35% of the total content, which includes water.

When we look at the chemical structure of blood, we see Fe^{+2}, N, cyclic groups but not aromatics, CH_3, CH_2 and COOH

No Aromatic group.

1st Iteration of CP observations:

We are using a current of -20mA. Voltage output over the time period is on average ~ 3V.

It requires ~ 1½ hrs for full separation to take place. We have three distinct layers forming:

1. Foam - present on the syringe. The vial is ~ ½ ml of distilled water w/ ~ 3-4 drops of blood contained within. The foam layer is ~ ⅓" thick (1-1.5 cm). There are some very minor traces of blood within the foam layer. It is primarily an off white foam layer.

2. The bulk of the vial solution (~80-85%) becomes transparent w/ a slight reddish tinge to it. All electrodes have some level of gaseous activity present.

3. A dark red layer (presumed to consist primarily of red blood cells) is on the bottom of the vial. It's appearance is consistent with blood (visually) but it is a darker, reddish red color than when first placed into the vial, as when it is bright red. The layer occupies ~15% of the volume of the vial.

I intend to collect representative samples of these 3 layers and inspect them under the scope.

I would then like to, if possible, further subject each layer to a centrifuge process.

I have the separations collected. I will now look and record under the microscope.
Magnification will ingeneral range between 400-1600x

Ok, the top foam layer has been reviewed. It is no secret what is happening here. Low power, eg 400, is more than sufficient to identify to characterize the layer. There are 3 primary components

1. Advanced filament presence
2. CO3 abundance
3. Early stages of filament production
4. Material which appears to be that of a protein.

There IS NOT any layer that is domenated by blood cells or the color of red. No actual blood cell structures, types or shapes are ever visible in the top layer separated analyses.

The remains as the primary target of the entire process that has been developed. Full analysis of the layer is

verified by all means available to,

1. Protein detection
2. NIR profiling
3. Electrochemical profile
4. Electrochemical Isotopic analysis.

This has all been previously done, however must shall repeat the process to the level of certainty desired.

---

* Now I have examined the bottom layer which was presumed to be dominated by blood - red blood cells. It is not blood in any conventional sense of the word. There is a complete transformation of all "blood" that has taken place.

I have seen a phenomenon like before, but it was somewhat dismissed as some anomaly. It is not an anomaly as will be described.

There are only a few isolated instances of anything that could be even interpreted as a blood cell.

What the massive layer on the bottom (which incidentally remains a deepred, but retains a darker red than ~~for~~ fresh blood is.) is composed of is:

1. A very high number of fully developed filaments.

2. There a protein structure that seems to be present, but it is COB based, not red blood cell based. It is a massive & fairly uniform transformation of the nature of the blood.

It really can not be called "blood" in any conventional understanding.

It is an entirely different protein construct that is compared to a COB fundamental basis, and it is enmeshed with numerous advanced fully developed filaments.

And now not we have another unusual development, and the subtle "anomaly" referred to:

The new protein forms into "band" structures. Not only that, they "bands" or "relations" of proteins (I will give first estimate, not measured, of maybe ~30 um in width)

* ALIGN themselves parallel to one another on the microscope.

I have seen this before but now it is extremely well documented.

This would be most readily interpreted as

"aligned" or "Parallel" Coagulation of the particular proteins.

If there is a known alignment, it would seem as though the longer axis of the bands are aligned in E-W orientation, but this will have to carefully determined.

Either way, what is apparent here is massive and radical transformation of the blood as well as a systematic pattern of coagulation taking place.

* It is extraordinary

The mass of the blood sample is almost entirely
* accounted for (with the exception of the clear largely transparent base solution) with this analysis.

"
THERE IS NO CONVENTIONAL" blood analysis
* that can take place after the transformation has been made via the application of an electric current through the blood.

This is quite phenomenal. At this point, there may or may not be a need for successive separation, but we will see.

And now for the last layer, which we referred to as the base solution. The solution is transparent by eye, w/ no visible solid components, and has a slight pinkish tinge.

Under the microscope, the layer comes out as non eventful, with no materials of any kind visible. We assume something of some form exists due to the very slight pinkish color, but it would seem to be either water soluble, or of low concentration a such.

Of all three layers, the layer of greatest interest and mass is the settled layer, the "transformed, parallely aligning, coagulated protein".

It is now suitable to proceed to protein detection tests for all three layers. It is reasonable to presume at this stage, that hemoglobin based protein in a conventional sense, will no longer actually characterize these samples. The presumption will be treated w/ great caution, but there is good cause to put forth the proposal even @ this early stage of analysis.

Next:

We take on protein detection of the three individual layers.

Now we call that it must be water soluble to conduct the test.

1. Our "foam" sample was extremely small and at has dried up by now. I have added about 3-4 drops of H_2O to it. Destilled water always. It can be seen that this solution contains insoluble in it. Filaments and the observed protein - CO3 would be as expectation here. I will add 10 ml of a dilute KOH - NaOH solution to it to see if it ~~collagen~~ can get it dissolved w/ maximum dilution to it.

Dilute KOH - NaOH is 1 drop in 1.5 - 2 ml of H_2O. Added added 20 ml of the dilute KOH - NaOH. It was not strong enough to produce a change in solubility, as indicated by the shift to green when solubility occurs

I have then added 10 ml of full strength (~10M) KOH - NaOH. The has gradually produced the desired effect, the solution is of green tint and it appears to have introduced sufficient solubility to proceed with the protein detection test.

My total sample size on the layer is now (foam layer). ~0.5 ml

Spectrometry: VIS'

Calibrate the reference to be the protein reagent.

The protein reagent is now a full orange color, no longer red. It seems to have no impact upon its ability to detect a protein change color shift. The reagent is now about 3 weeks old and has been stored w/in a black sock.

There are immediate significant and pulsating peak absorbance that appear in the spectrum when layer 1 - the foam - CDB layer is added (already observed & contains filaments & suspected protein content). Then all 30 µl added to vial.

* The test is thus far highly positive for protein content. Here are our spectrum peaks.

There are major peaks. Spectral change on a smaller scale also occurs:

LAYER 1 - FOAM PRECIP

λ	Abs (5min)	Abs (15min)	The reference spectrum remained perfectly flat prior to protein introduction
383 nm	0.130	.149	
409 nm	.061	.070	
424 nm	.057	.066	
906 nm	.050	.056	
932	.079	.090	

These match those of previous trials, including that of Carson.

So we therefore have a highly positive test results for the existence of protein within the foam-prep layer separated by Chromopotentiometry and also subjected to microscopic examination and recordy.

In the ideal world you would like to add NIR and ACV analysis but I may not have sufficient sample. We shall see.

—

Now lets go on to layer 2 which will be the settled red (darker red) layer which was also extensively examined and recorded under the microscope. The analysis indicates this to be a completely transformed protein from that of blood. Refer to the extensive notes and description of that matter.

Now you know the same issue of solubility is the case. You are required to exploit Na-KOH to ensure solubility. We will do so at the level that introduce the green color shift, which indicate the presence of Fe+2 hydroxide.

Start w/ 20 gtt KOH-NaOH. The appear to be sufficient.
50 ml

The dark red is so strong that it has become even darker, but the material is now dissolved.

Protein reagent as the reference.
Later add 30 ul to 1 ml.

The reference spectrum was again perfectly flat prior to protein introduction.

The color of the sample is indeed a dark red - olive green combination. I have plenty of sample available here.

Concentration in the sample seems to be quite high (as expected) w/ strong peaks indicated.

λ	Abs (5 min)	Abs (15 min)
384 nm	0.188	.203
405	.103	.115
901 nm	.051	.058
939	.095	.109

The 424 peak seems to be engulfed within the strong peak @ 405 nm.

Therefore the settled layer, which comprises the vast majority of the mass collected after CP clearly possesses the protein of interest.

* Notice that no centrifuging was required to further distinguish, separate & collect these layered samples. An inserted pipette at the proper depth is sufficient.

And now for the third layer which we may called the bead layer. 100 ul then beaded to the reagent vial since it is presumed to be of much lower concentration. I have plenty of sample.

Spectral analyses indicate exactly as above. The peaks are generally less well defined except for C 303. That is always an interesting peak here. The results are:

λ	Abs (5 min)	Abs (15 min)	
384 nm	0.138	0.129	
400	.088	.074	(almost a shoulder peak)
908 nm	.029	.030	
939	.050	.052	

The test is also, however, positive for protein detection. The concentration level is undoubtedly much lower here. Also, the character of this protein "version" would say that it is much more soluble than the other.

We have, therefore, potentially 3 different varieties of protein that resulted in three bands separated as a result of the electrochemical process applied (CP).

My methods of sample prep, processing, collection and analysis of data have become much more efficient than when I started the project.

At this point, no iterative electrochemical process now seems to be required, although it still may be of interest to explore.

We actually now have 3 separate distinct versions of protein to analyze; top, middle & bottom layer of the CP processing. No centrifugation has now been required.

Since I have so little sample material of the top foam-precip layer (now known as protein in content) I have gone ahead and started the electrochemical profile collection.

The method for inorganic analysis will be ACV (AC Voltammetry) furthermore with separation of both AC & DC components.

I have placed 50 ul of Layer 1 Protein into the electrochemical cell of ~4 ml volume distilled H_2O w/ graphite electrode.
I will collect some of that primary data tonight.

Apr 27 2022

The electrochemical ACV profile of Layer 1 - Foam Precip protein is showing a great deal of variable activity, even after 19 runs.

NIR Scans:

Layer 1 - Foam Precip:

1323 Peak

1649 Peak

1535 Ascending Inflection

1569 Ascending Inflection

925-930 Peak

1389 Descending Inflection

1205 Descending Inflection

We must set the Reference Scan to New every time
Never use Previous.

NIR Scan Layer 3 - Settled Layer

920 peak

1330 Descending inflection

1398 Descending inflection

1522 – 1528 Ascending inflection

1565 – 1570 Ascending inflecti

1648 Peak

1109 Descending inflection

946 – 954 Minor Peak

Now the middle layer, the "liver" layer

1321 descending inflection
1397 descending inflection
1645-1651 Peak
1526 Minor Peak
1426 Descending inflection, peak

935 Peak

Moreover information is available on the spectrum. The cuvette w/ expected low concentration.

An observation:

I noticed now that the "base solution" formerly regarded as being clear and that no K-Na OH was added but in the protein detection ~~test~~ 24 hrs later I now see a precipitate that exists within the base solution. It may therefore be worthy of repeating my analytical tests, ie

1. protein detection test
2. NIR analysis
3. Electrochemical ACV profile (actually not done yet)

after some K-Na OH has been added & expected to turn green.

I want to back up a little bit here to the "blank" layer, that which has now shown precipitates to form in what was presumed to be a "transparent" pink colored solution.

Because of the new precipitation observed I have added 30 ml of KOH-NaOH. This does have the effect, as expected, of dissolving the precipitate and now turns the solution olive green, also as expected.

Now we will back up and repeat the VIS protein reagent test to see if we now get a stronger signal therein.

I will then repeat the VIS spectrum. I have added the 50 ml of the now dissolved sample into the reagent reference vial

λ	Abs (15mm)	Notes
382nm	0.156	And we see that there is a match to the spectrum previously recorded but with stronger signal as anticipated.
409nm	.082	
907nm	.068	
936	0.104	

Protein Extraction test is highly positive
Reference spectrum measured just prior to the introduction of the protein.

What we see here is that the settled layer and the bulk layer record essentially the same spectrum. The says nothing about the protein equivalency, however, as only VIS spectrometry is used here.

The deconstruction of the splitting of the 409 peak region into two separate peaks, ie 409 & 424 nm for the foam precipitate protein, however puts us on notice to be looking for any chemical change that might be detectable w/ our available instrumentation.

The was important to regroup on & repeat

Now we can once again proceed w/ electrochemical profiling via ACV on the various layers.
We have now recorded the foam-prep protein from -3.5 to 3.5V and reverse from 3.5V to -3.5V.

I would like to look the profile of the foam prep protein from -1.5V to 1.5V and in reverse to see if I can pick up any additional information here.

I have adjusted EStep from 0.02 to 0.01 to double the sample number.

Actually I am picking up a couple of prominent peaks w/ the ACV profile, not the DC so there likely is new information coming in.
Let's continue w/ @ least 10 scans in each direction.

Ok, the look to be decent data collection.

Now let's go on to the settled layer. 50 ul added
-3.5V to 3.5V and 3.5 to -3.5V
Electrochemical profile collected.

Now to "base" layer profile. 50 ul added

May 01 2022 Exclude K, Na

We now have a large body of data collected both in electrochemistry and NIR
Lets go with round 2 analysis to see what we learn.

First case will be ACV on the foam pencil protein layer.
I have ~24 scans available
$n = 0$

$y^\circ(ACV)n=0$

- ~~0.83 Peak H_2O, H^+ (-.83)~~

✓ - 0.50 descending inflection (-.44 Fe^{2+})

✓ -1.76 peak H_2O_2 (1.78)

✓ -1.65 peak Al(-1.68)

✓ -1.50 peak Cl(1.46) Mn(1.51)

✓ -1.44 desc. infl. Cl_2(1.46)

✓ -1.03 des. infl Br(1.07) NO_3^-(.96)

✓ +2.42 peak Mg(2.36)

$y^\circ(OC)n=0$

+3.03 peak ~~Li(-3.04)~~ K(-2.93)

✓ +1.33 asc infl Cl_2(1.36) Cr(1.33)

✓ -1.06 asc infl Br(1.07, 1.09)

✓ -2.00 asc. infl. (minor, ?) Mg(2.02)

✓ -2.42 peak Mg^{2+}(2.36)

$n=5$: Exclude Na, K)

$y°(ACV)$ $n=5$	$y°(OC)$ $n=5$
✓ −3.37 peak (3.34 HN_3)	✓ −3.36 peak (HN_3 3.34)
−1.71 peak (Al, 1.68) H_2O_2 (1.78)	−2.86 (minor peak) Ca (2.87)
−1.56 peak Mn (1.51) Cl (1.63)	~~−2.75 (minor peak) Na (2.71)~~
✓ −1.44 peak Cl (1.46)	✓ −2.36 peak minor Mg (2.36)
✓ −1.36 peak Cl_2 (1.36)	✓ −2.32 peak (Mg, 2.32)
(?) ?? −0.36 peak (broad) CH_3OH (0.24)	✓ −2.18 (peak minor) Fl (2.17)
~~?~~ ✓ 2.11 desc. infl, Fl (2.17) ? (twice)	−2.00 asc. infl, Mg^{2+} (2.02)
	I_2 (1.21) −1.12 asc. infl (broad) Br (1.09)
	✓ 1.66 asc infl, Al (1.68)
	~~2.63 peak Na (2.71)~~

n=10 Excl Na, K

	4° (ACV)	4° (OC)
	-3.36 ($3.34\ HN_3$)	-3.42 ($3.34\ HN_3$)
(?) ??	-1.88 asc. infl. NH_3OH^+ (1.83) Al, Cl (1.80)	2.58 peak Mg (2.69) H_2O (2.58)
✓	-1.76 peak (H_2O_2 1.78)	-1.76 asc. infl. (H_2O_2, 1.78)
✓	-1.56 peak Mn (1.51) Cl (1.63) [1.63]	
✓	-1.44 peak (Cl, 1.46)	
✓	-.93 asc infl NO_3^- (0.96)	
✓	-0.52 asc infl. (I_2, 0.54)	
(?)	-0.25 desc. infl SO_4^{2-} (0.17) CH_3OH (0.24) Fe^{2+} (glu-lemole) 0.24	
	2.04 desc. infl Mg^{2+} (2.02)	

n=15 Exclude Na, K

4° (ACV)

?? -2.12 asc. infl. Fl (2.17)

-2.02 minor peak Mg (2.02)

-1.98 minor peak Mg (2.02)

(?) -1.84 asc. infl. NH_3OH^+ (1.83) $AlCl$ (1.80)

-1.59 asc infl. Cl (1.63)

-1.44 asc. infl. Cl (1.46)

-1.37 asc. infl. Cl (1.36)

known from Fe+2 grow -0.44 des. infl. (Fe^{2+} (.44)

-0.51 desc infl. I_2 (0.54)

+2.44 des. infl. ~~Mg (2.36)~~ ~~$Mg(C_2O_4)_5$ 2.49~~ H_2O (2.43)

4° (OC)

(?) ?? -3.16 desc. infl. K is closest [2.93] exclude

?? -3.14 desc infl

-3.00 asc. infl K (~~2.93~~)

-2.96 peak F_2 (~~2.89~~) K (2.93)

-2.80 peak ~~F_2 (2.89)~~ Ca (2.86)

-2.74 peak Mg (2.69)

H_2O (2.43)

-2.48 asc. infl. ~~$Mg(C_2O_4)_5$~~ ~~Mg (2.49)~~

-2.30 asc. infl Mg (2.36)

(?) -1.25 asc infl (broad)

-2.81 asc. infl. F_2 (2.89) Ca (2.86)

N=20 Exclude K, Na from solvent use_

4° ACV	4° DC
✓ 3.44 (sharp peak) HN_3 (3.34)	~~3.28 Peak HN_3~~
✓ ~~2.87 asc. infl. Ca (2.86)~~	✓ ~~2.88 desc infl Ca (2.86)~~
~~2.43 asc. infl. H_2O (2.43)~~	~~2.44 asc infl H_2O~~
✓ ~~2.26 asc infl. Fl (2.17) Mg (2.36)~~	✓ ~~2.32 asc. infl Mg (2.36)~~
✓ ~~2.22 asc infl Fl (2.17)~~	
~~2.18 asc infl Fl (2.17)~~	Ø.30 mild asc infl
✓ ~~2.16 peak Fl (2.17)~~	~~O_2 (.41)~~
	✓ 2.67 peak Mg (2.69)
✓ ~~2.04 asc. defl. Mg (2.02)~~	
~~1.92 peak K (1.98)~~	
(?) ~~1.84 asc. infl. H_2O (1.76) NH_3OH (1.83)~~	
~~1.78 H_2O (1.76)~~	
✓ ~~1.60 asc infl Cl (1.63)~~	
✓ ~~1.46 asc. infl Cl (1.46)~~	
.005 desc. infl. H_2	
+.01 asc infl HO_2 (.05) Notice! PH_3 (.05) H_2 (.00)	
CH_4 (.03)	
Ø.60 desc infl. Mn (Ø.60) I_2 (Ø.62) SO_3^{2-} (Ø.66) Br (Ø.61)	
CH_3OH (Ø.58) I_2 (Ø.54) MnO_4^- Ø.52 H_3PO_2 (Ø.51)	
H_2SO_3 (Ø.57) SO_2 (Ø.54) SO_3^{2-} (Ø.57)	
✓ ~~2.19 peak Fl (2.17)~~ Strong candidate, no competition, strong peak	
✓ ~~2.27 desc infl · Mg (2.36)~~	

$S = \ln\left(\frac{n}{\bar{x} \cdot \sigma_s}\right)$ $n\sigma_s = 0$ $S = \ln\left(\frac{n}{\bar{x}}\right)$

Ok, I think that we have enough information to answer the foam-prep for protein primarily inorganic nature of our ACV profile.

This analysis is based upon y° use only not the derivative.

Absolute values

$n=2$ $\bar{x}=.030$ $\sigma_s=.042$ Fe^{2+} [.44] .50, .44 7.37

$n=3$ $\bar{x}=.013$ $\sigma_s=.012$ H_2O_2 [1.78] 1.76, 1.78, 1.76 9.86

$n=3$ $\bar{x}=.027$ $\sigma_s=.006$ Al [1.68] 1.65, 1.71, 1.66 9.83

$n=13$ $\bar{x}=.03$ $\sigma_s \simeq .022$

$n=6$ $\bar{x}=.010$ $\sigma_s=.011$ Cl [1.46] 1.50, 1.44, 1.46, 1.46, 1.46, 1.46 9.88

$n=4$, $\bar{x}=.0275$ $\sigma=.026$

$n=3$ $\bar{x}.037$ $\sigma_s=.023$ Mn [1.5] [0.60] 1.50, 1.56, 1.56 Ø.60 8.63

$n=8$ $\bar{x}=.023$ $\sigma_s=.02$

$n=4$ $\bar{x}=.022$ $\sigma=.013$ Br [1.07] 1.03, 1.06, 1.09, 1.09 9.76

$n=2$ $\bar{x}=.05$ $\sigma_s=.028$ NO_3^- [Ø.96] 1.03, Ø.93 7.26

$n=14$ $\bar{x}=.035$ $\sigma_s \simeq .02$

$n=8$ $\bar{x}=.045$ $\sigma_s=.033$ Mg [2.36] 2.42, 2.42, 2.36, 2.32, 2.30, 2.26, 2.27, 2.32 9.90

~~$n=3$ $\bar{x}=.03$ $\sigma_s=0$ Cl [1.33] 1.36, 1.36, 1.36 4.60~~

~~$n=4$ $\bar{x}=.025$ $\sigma_s=.026$ Br [1.09] [0.61] 1.03, 1.06, 1.09 .60 8.72~~

~~$n=6$ $\bar{x}=.023$ $\sigma_s=.008$ Mg [2.02] 2.00, 2.00, 2.02, 1.98, 2.04, 2.04 10.39~~

$n=4$ Cl [1.63] 1.56, 1.63, 1.59, 1.60 8.28

$\bar{x}=.035$ $\sigma_s=.029$

$\bar{x}=.046\ \sigma=.03$ $n=5$ HN_3 [3.34] 3.31, 3.36, 3.36, 3.44, 3.28 — 8.07

$\bar{x}=.037\ \sigma=.030$ $n=8$ Fl [2.17] 2.11, 2.18, 2.12, 2.26, 2.22, 2.18, 2.16, 2.19 — ~~8.88~~ (8.88)

$\bar{x}=.03\ \sigma=.023$ $n=5$ Ca [2.86] 2.87, 2.80, 2.81, 2.87, 2.88 — 8.89

$\bar{x}=.040\ \sigma=.034$ $n=4$ I [1.21] 1.12 — 7.99
[.54] .52, .51
[.62] .60

~~Br [0.61] 0.60~~

~~Mg [2.69] 2.74, 2.67~~

~~Mn [0.60] 0.60~~

SO_2 [0.54) 0.60 — 2.81

SO_3^{2-} [0.57) 0.60 — 3.51

These are our ranks are:

Mg	9.90	Ca	8.89	SO_3^{2-}	3.51
Cl	9.88	Fl	8.88	SO_2	2.81
H_2O_2	9.86	Mn	8.63		
Al	9.83	HN_3	8.07		
Br	9.76	I	7.99		
		Fe	7.37		
		NO_3^-	7.26		

Inorganic Summary – Foam. Precip Problems

* * *

The results of the analyses indicate that the foam/precip inorganics may consist of

1. Halogens — Cl, Fl, Br, I
2. Peroxide (oxidizer)
3. Nitrogen Mustard
4. Electrolytes
5. Metals such as Iron, Aluminum & Manganese
6. Nitrogen and sulfur compounds

As I recall, the analyses matches over [illegible] new analyses essentially to a tee.

The second study was completed on a repeat [illegible] from the ground up and they are further confirming the likelihood of the [illegible] they have.

May 02 2022

Let look @ the NIR data recorded for the foam precip protein on Apr 27 2022. We have 4 following peaks or inflections.

1323nm, 1648, 1535, 1569, 925-930, 1389, 1205

These are dry samples, H_2O should not be a factor

925-930. CH_2(3), CH_3(1), CH(2) ROH(2)

1205 CH_2(2), CH(3), CH_3(1)

1323 No Match - This was a peak

1389 CH_3(3), CH_2(2), ArOH(2)

1535 RNH_2(1) No Competition

1569 No Match, No Competition

1648 ArCH(2), CH_3(2) CH_2(1)

Notice the overlap & repetition of assignments. Like w/ the notes of Apr 19 2022

Scoring:

CH_2 (3+2+2+1) = 8

CH_3 (1+1+3+2) = 7

CH (2+3) = 5

ROH = 2

ArOH = 2

$ArCH_2$ = 2

RNH_2 = 1

ROH ⇒ 4

Ar ⇒ 4

No Competition

Including consideration of halogen substitution we most likely have each of the functional groups present.

CH, CH_2, CH_3, RNH_2, ArOH, $ArCH_2$.

Organic Summary – NIR
Foam – Precip Protein

In addition, we call and notice our repeated VIS scans involving protein detected @ ~907 nm & 937 nm.

The observation was analyzed on Apr 22 2022 and also compared to a reference study of Casein protein in milk.

The functional groups attached to these absorptions are:

907 $CH_2(3)$, $CH_3(2)$, ROH(2)

937 CH(3), $CH_2(2)$ ROH(3), ArOH(1)

The bonds are scores to.

CH_2: 8 + 3 + 2 = 13
CH_3: 7 + 2 + 5 = 14
CH: 5
ROH: 4 + 5 = 9
Ar: 4 + 1 = 5
ArOH: 2 + 1 = 3
ArCH: 2
RNH_2 = 1 → ∞ No Competition.

We have completely consistent results with the prior analyses made.

Halogen substitution almost a certainty

There are relatively high scores for each functional group. A presumption can be made that these functional groups exist w/in the protein

CH, CH_2, CH_3, ROH, RNH_2, Ar, ArOH, ArCH

These are to be combined w/ the inorganic analyses

Our next question of importance is whether we can distinguish any difference of note between the foam-prep protein & the pelleted-prep protein

May 05 2022 Excludes Na, K

Now I will proceed to the "settled layer" proteins.

An observation is that the foam-precip protein electrochemical profile seems to be more noticeably more complex and active than the settled layer. It will be curious what the analysis shows.

-3.5 to 3.5V n=10 Scans

ACV 4°	DC 4°	ACV 4'	DC 4'
-2.86 desc. infl.	-3.12 asc. infl.	.58 peak	-3.46 peak
Ø.12 asc. infl (minm)	-.007 asc infl	Ø.29 peak (small)	2.80 peak
Ø.65 asc. infl	-3.33 asc infl	Ø.93 peak	3.30 peak
1.66 peak	.003 asc infl.	-Ø.16 peak	Ø.37 peak
1.83 peak	2.14 asc infl.	.65 peak	
Ø.46 peak	3.06 asc infl.	1.82 peak	
Ø.76 peak		3.36 asc. infl.	
.65 peak		-.005 peak	
2.46 asc. infl		-3.02 asc infl.	
		Ø.84 peak	
		+2.30 peak	

AC & DC components

Settled Layer Protein 3.5V to −3.5V ACV. n=13

ACV 4°	ACV 4'	
✓ +2.88 peak	✓ Ø.34 peak	✓ Ø.10 peak
		✓ 2.75 peak
✓ +1.43 peak	✓ 2.63 peak	✓ 2.80 zero
		✓ Ø.12 asc. infl
associate w/ Ca 2.93 desc infl.	✓ 2.87 zero cross	✓ 2.74 peak
✓ +3.04 desc. infl	✓ −.005 peak	
✓ 3.33 desc. infl	✓ +1.80 peak	
✓ +1.40 peak	✓ +1.46 zero cross	
✓ 2.81 desc. infl	✓ +2.50 peak	
✓ Ø.28 asc. infl	✓ + 3.42 peak	
✓ 2.89 asc. infl	✓ +.008 peak	
✓ 3.18 desc. infl	✓ 2.37 peak	
	✓ 2.64 peak	
	✓ +1.41 zero cross	
	✓ +.10 asc. infl	
	✓ + 2.36 peak	
	✓ 2.64 peak	
	✓ 2.96 zero	

Settled Layer Pirotech — Inorganic Analyses

2.86, 2.80, 2.88, 2.81, 2.89, 2.87, 2.96, 2.80, 2.74, 2.75

SPLIT:

2.86, 2.88, 2.89, 2.87, 2.96 (.03, .05, .06, .04, .13, .03, .02, .03, .11, .08)

2.80, 2.81, 2.80, 2.74, 2.75

Combined mean = 2.83 Ca [2.86] n=10, $\bar{x}$=.06 σ_s=.04 S= 8.33

(.07, .02, .07, .01) .31, .46, .84, .76 $\left[\frac{Fe^{+2}}{Fe^{+3}}\right]$ .44, .77 S= Score = $\ln\left(\frac{n}{\bar{x} \cdot s}\right)$ = S = 8.05

n=4 $\bar{x}$=.04 σ_s=.032

0.12, 0.16, 0.10, 0.12 ($\bar{x}$=0.12) SO_4^{2-} [0.17]

(.05, .01, .07, .05) n=4 $\bar{x}$.045 σ_s=.025 S= 8.18

0.65, .65, .65, .58 ($\bar{x}$=0.63) Br [.061] is best match S=9.97

Br [0.61] (.04, .04, .04, .03) n=4 $\bar{x}$=.0375 σ_s=.005 S=9.97

Mn [.60] (.05, .05, .05, .02) n=4 $\bar{x}$=.04 σ_s=.015 S=8.80

1.66 Isolated n=1 (peak) Al [1.68] n=1 σ=.02 S= 3.91 w/ Peak

1.83, 1.82, 1.80 $\bar{x}$=1.82

H_2O_2 [1.78] n=3; (.05, .04, .02) $\bar{x}$=.04, σ_s=.015 S= 8.52

2.46, 2.50, 2.37, 2.36 n=4 Mg [2.36]

(.10, .14, .01, 0) $\bar{x}$=.06 σ_s=.07 n=4 S= 6.86

3.12, 3.06, 3.02, 3.04, 3.18 $\bar{x}$=3.08 K [2.93] Exclude K

.007, .003, .005, .008, .10, .005, .10 Accept as H_2

3.33, 3.36, 3.46, 3.30, 3.33, 3.42 $\bar{x}$=3.36 HN_3 [3.34]

(.01, .02, .12, .04, .01, .08) n=6, $\bar{x}$=.05, σ_s=.04 S= 8.01

2.14, 2.30 These do not justify a separate entry @ this point and are most likely to be associated w/ Mg of [2.36]

2.63, 2.64, 2.64 $\bar{X}$=2.64 Na[2.71] Exclude Na

.29, .31, .34, .28 $\bar{X}$=0.32 This could be associated w/ O_2 @ [0.41] but a bit low

@ the point could be associated but may not justify a separate entry, however we do have

$Fe(CN)_6^{3-}$, $Fe(CN)_6^{4-}$ @ [0.36]

And there is a real possibility consider our previous identification of isothiocyanate in blood via IR. It is a candidate.

also $Mn(OH)_2$ [0.35]

Phosphorus H_3PO_3, H_3PO_4 [0.30]

Sulfur S^{2-} [0.34]

We do therefore have an additional Candidate here,

1.43, 1.40, 1.46, 1.41 $\bar{X}$=1.42 Cl_2[1.36]

(.01, .04, .09, .05) n=4, $\bar{X}$=.06 σ=.02 S= 8.11

Scores:

✓ Fe	8.05
✓ Ca	8.33
✓ SO_4^{2-}	8.18
✓ Br	9.97
✓ Mn	8.80
Al	3.91
✓ H_2O_2	8.52
✓ Mg	6.86
✓ HN_3	8.01
✓ Cl_2	8.11

Next we Rank:

Br	9.97
Mn	8.80
H_2O_2	8.52
[H_3PO_4]	8.39 (new candidate)
Ca	8.33 → SO_4^{2-} 8.18, Cl_2 8.11
Fe	8.05
HN_3	8.01
[$Fe(CN)_6^{-3,or-4}$]	7.84 new candidate
Mg	6.86
Al	3.91

Additional Candidate of H_3PO_4 or $Fe(CN_6)^{-3,or-4}$

Score for H_3PO_4: [0.30]

(.01, .07, .04, .02) $n=4$ $\bar{x}=.035$ $\sigma_s=.026$ $S=8.39$ ✓

Score for $Fe(CN)_6^{-3,or-4}$ [0.36]

(.07, .01, .02, .08) $n=4$ $\bar{x}=.045$ $\sigma_s=.035$ $S=7.84$ ✓

So add

H_3PO_4, H_3PO_3	8.39
$Fe(CN_6)^{-3,or-4}$	7.84

Inorganic Analysis - Comparison of Foam Precip Protein ⟺ Settled Layer Proteins

Now let's compare the foam-precip protein with the settled layer proteins, underlyside (Ranked)

Score	Foam Precip Protein		Settled Layer Protein	Score
			Br^-	9.97
9.90	Mg			
9.88	Cl^-			
9.86	H_2O_2			
9.83	Al			
9.76	Br			
8.89	Ca			
8.88	Fl			
			Mn	8.80
8.63	Mn			
			H_2O_2	8.52
		new Candidate	$[H_3PO_4]$	8.39
			Ca	8.33
			SO_4^{2-}	8.18
			Cl_2	8.11
8.07	HN_3			
			Fe	8.05
			HN_3	8.01
7.99	I			
		new candidate	$[Fe(CN_6)^{-3\text{ or }-4}]$	7.84
7.37	Fe			
7.26	NO_3^-			
			Mg	6.86
			Al	3.91
3.51	SO_3^{2-}			
2.81	SO_2			

Now for some comments on the comparison.

Ranking CANNOT be interpreted as a probability, only as a statistical strength-confidence indicator within that particular protein solution data set. Do not attach special significance to the various ranks, but it can be considered as an indicator of statistical "strength" of existence.

What is important is to look @ the degree of overlap or coincidence of inorganic elements or structures identified. These show themselves to be remarkably identical even though totally separate sample types were used, and the electrochemical analyses were entirely independent of one another.

Then mean that the elements or structures listed, IN BOTH CASES, are VERY STRONG CANDIDATES for existence w/in the two separate proteins. The separation is by density, the foam precip less dense & the settled layer more dense.

We must also note that two very important new candidates exist w/in the settled layer. These are the consideration of either a both of ferrocyanide and phosphoric acid compounds. Examination of the literature record by research will show either there is a case to be made for the existence of both. All elements and compounds are to be regarded as likely constituents of the proteins.

NIR Organic Analysis of "settled layer protein

Next we need to start Complete a NIR analysis of the "settled layer" protein.

From the notes of Apr 27 we have the following:

920 peak $CH_2(3)$ $CH_3(1)$ $CH(1)$ $ROH(2)$

1330 dec. enfl No match

1522-1528 arc. enfl $RNH_2(2)$ No Competition

1565-1570 arc. enf. No match

1648 peak $ArCH(3)$ $CH_3(2)$

1109 dec. enf $ArCH(1)$

946-954 Mon. peak. $ROH(3)$ $ArOH(3)$

Recall we also have w/ the protein reagent the following additions

907 $CH_2(3)$ $CH_3(2)$ $ROH(2)$

937 $CH(3)$, $CH_2(2)$ $ROH(3)$ $ArOH(1)$

Scores are

CH_2 (3) $+3+2 = 8$
CH_3 $1+2+2 = 5$
CH $1+3 = 4$
ROH $2+3+2+3 = 10$
RNH_2 (2) No Competition
$ArCH$ $3+1 = 4$
$ArOH$ $3+1 = 4$

Rank:

RNH_2 No Competition $= \infty$
ROH 10
CH_2 8
CH_3 5
CH 4
$ArCH$ 4
$ArOH$ 4

Organic Comparison of 2 Protein phases or forms:

Next, let us place the foam precip organic analysis alongside the settled layer protein for comparison:

Score	Foam Precip Protein		Settled Layer Protein	Score
∞	RNH_2 (8)	NO COMPETITION	RNH_2 (8)	∞
14	CH_3 (1)			
13	CH_2 (2)			
			ROH (3)	10
9	ROH (3)	Exact match in functional group identity w/ similar reactivity levels		
			CH_2 (2)	8
			Ar (5)	8
5	CH (4)		CH_3 (1)	5
5	Ar (5)			
			ArCH (7)	4
			ArOH (6)	4
			CH (4)	4
3	ArOH (6)			
2	ArCH (7)			

Therefore we have a complete coincidence of functional groups in both protein cases.

In general, the two phases or forms of protein collected appear to share fundamental organic and inorganic components in a fairly equivalent fashion.

The would seem to provide sufficient information as to what characterizes these two proteins from one another,

given the means available to be able to analyze them.

The analysis of the remaining layer, the "base" solution may not be of high priority @ this time. Let me look @ the electrochemical profile complexity levels:

The predominant activity of the electrochemical activity of the remaining layer appears to center around + 3.0V

We know that the range encompasses largely the electrolytes of Na, K, Ca, etc

Thus should not be any real surprise that dissolved electrolytes would form a core component of the layer.

We do also know that the "base layer" does also test positive for protein.

I will allow the information to suffice for the time being w.r.t. inorganic analysis of the protein from.

The NIR spectrum data also does not appear to provide any substantively new or different functional group data so we also will be allowed to suffice for now.