CARNICOM INSTITUTE LEGACY PROJECT

A Release of Internal Original Research Documents

Authored
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Chemistry
Vol 2

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5 SUBJECT
180 Sheets
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Chemistry Notebook

Feb 07 2010

vol 2

Successful Culture Mediums have now been developed:

White Wine
10 drops $FeSO_4$
Filament (or lye reduced form)

~~Artificial~~
Simulated Wine
1. Acetic Acid 25 drops 28% (1.25 ml)
2. Alcohol 48 ml
3. Water 330 ml
4. Fructose 8 gms
5. Salt 1.2 gms

Now we calibrate pipette:

80 drops = 4 ml

$\frac{80 \text{ drops}}{4 \text{ ml}} = \frac{1 \text{ drop}}{x}$ $x = .05$ ml

also, 1 teaspoon = 4.93 ml $\tilde{=}$ 5.0 ml

If we want to use vinegar instead @ 3%

$\frac{28\%}{3\%} = 9.33$ $9.33(25 \text{ drops}) = 87.1 \text{ drops}$
$= 4.35$ ml
$\tilde{=}$ 1 teaspoon of vinegar

We have some test results:

Non Productive	Moderately Productive	Productive
Acetic "Wine" + Filament		White Wine + Filament + $FeSO_4$
White Wine + Filament		White Wine + Filament + $FeSO_4$ + H_2O_2 (most productive)
HCl + Lye + $FeSO_4$ + H_2O_2		
HCl + Lye + $FeSO_4$		
Acetic Wine + Filament + $FeSO_4$		
Isopropyl "Wine" + Filament		
HCl "Wine" + $FeSO_4$		
HCl Wine + Filament		
Filament + Sugar		
White Wine + $FeSO_4$ + Transferred Wine Culture	"Acetic Wine" + Lye Culture + $FeSO_4$ + H_2O_2	
	White Wine + Filament	
	White Wine + Lye Culture + $FeSO_4$ + H_2O_2	
	White Wine ONLY + $FeSO_4$???? = ???	

So the question is, what is going to kill or inhibit this culture?

We know that

$FeSO_4$ feeds it
H_2O_2 feeds it
Acid feeds it.

What type of Chemistry is going on here that it likes & how do we threat it..?

Recall what we studied.
White wine does not contain very much Fe^{+2} or Fe^{+3}.
Red wine does test positive for Fe^{+2}
White wine fails the test for SO_4^-
Red wine fails test for SO_4^-
Confusing results for Conductivity of wine.
The successful culture fails the test for Fe^{+2} & Fe^{+3}.
Repeating test reveals slight detection of iron ions.

The successful culture & the white wine appears to test identically positive for SO_4. This tells us that the culture is not metabolizing SO_4.

Now what about iron?

White wine + 25 drops $FeSO_4$ we get a positive test for Fe^{+2}. (High concentration required)

Redesign test: Use same concentrations.

1. Wine + $FeSO_4$ (no culture) 1 day old
   2ml solution, 1 drop NaOH – no reaction
   2 drops – brown precipitate starts, but then dissolves
   5 drops – turns brown & stays brown.

2. Wine + $FeSO_4$ – fresh (10 drops)
   First off, when we mix well, we get what appears to be iron hydroxide in the bottom. But this may be in the $FeSO_4$ by itself first – it may not be any reaction at all.
   6 drops – identical brown color.

3. Successful culture (2 days old)
   6 drops NaOH turns brown identical color to Wine + $FeSO_4$ (no culture) 1 day old.

So we get the same results whether we are using the culture or Wine + $FeSO_4$.
(Wine + $FeSO_4$ + filament)
Strong producer.

We get a dark brown color.

The iron hydroxide may be a result of the $FeSO_4$ alone & it may not be producing anything in the wine.
Now my guess is that this iron is being oxidized.

$Fe(OH)_2 + O_2 \rightarrow ?$ maybe FeO_3???

Now when you are adding the peroxide, it really goes to town.

$Fe(OH)_2 + H_2O_2 \rightarrow ???$ FeO_3?

Now, this is when the wine flourishes.
Why? feeds off it?

What happens w/ acid & $FeOH_3$??

$Fe^{+2} + 4H^+ + O_2 \rightarrow 4Fe^{+3} + 2H_2O$

$Fe^{+3} + 3OH^- \rightarrow Fe(OH)_3$

These processes will be affected by pH.
(Because H^+ & OH^- ions are involved)

$Fe^{+2} + H_2O_2 \rightarrow Fe^{+3} + OH^- + \dot{O}H$?

(Fenton's reaction)

H_2O_2 is an oxidizing agent.

Ho radical is generated by Fenton's reaction

"Highly reactive hydroxyl radical (OH)

What are the ideas so far?

1. $CuSO_4$
2. Bleach
3. Baking Soda
4. Vit C
5. Anti Oxidants
6. MMS I
7. MMS II
8. Bak Soda + Anti Ox

It would be good now to be able to concentrate the bacterial form. Why?

A free radical prefers to steal electrons from the lipid membrane of a cell.

Something "radical" seems to have happened:

White Wine
$FeSO_4$
H_2O_2
Transferred Culture
+ Baking Soda!
+ (Anti Ox)

} seems to have caused an instantaneous explosion of growth ???

Bak Soda + Wine appears to generate CO_2.

White Wine
$FeSO_4$
H_2O_2
Baking Soda
+ Transferred Culture

Appears to produce an almost instantaneous explosion of growth.

White + $FeSO_4$ + Peroxide produces a reaction.
a white (ish) cloudy precipitate
+ Baking Soda
produces CO_2 in addition to above

so
produces OH radical

Wine + H_2O_2 + $FeSO_4$ + Baking Soda + Culture = Major Growth

Acid Oxygen Fe CO_2 + Culture = Major Growth
Sugar
Alcohol
Salt

MMSI is causing an even bigger reaction.
This is sodium chlorite $NaClO_2$
so
White + $FeSO_4$ + H_2O_2 + $NaClO_2$ + Culture = Major Growth.
Wine

Baking Soda + Water Reaction

$$NaHCO_3 + \overset{HOH}{H_2O} \rightarrow Na^{+1} + OH^- + H_2CO_3$$

$$H_2CO_3 \rightarrow CO_2 + H_2O$$

Fentons Reaction

$$Fe^{+2} + H_2O_2 \rightarrow Fe^{+3} + OH^- + HO^\circ$$

Now what happens with

$$Fe^{+2} + H_2O_2 + NaHCO_3 \rightarrow Na^{+1} + OH^- + CO_2 + H_2O$$
$$+ Fe^{+3} + OH^- + HO^\circ$$

Ok, we have solved the parts:

$$Fe^{+2} + H_2O_2 \rightarrow Fe^{+3} + OH^- + OH^\circ$$

$$NaHCO_3 + H_2O \rightarrow CO_2 + H_2O + Na^+ + OH^-$$

So

$$Fe^{+2} + H_2O_2 + NaHCO_3 + H_2O \rightarrow Fe^{+3} + OH^- + OH^\circ$$
$$+ CO_2 + H_2O + Na^+ + OH^-$$

yes this works, and simplifies to:

$$Fe^{+2} + H_2O_2 + NaHCO_3 \rightarrow Fe^{+3} + 2OH^- + OH^\circ + CO_2 + Na^{+1}$$

This reaction is what is different.

This creates major growth.

the difference between H_2O and CO_2 (and Na^+) in the presence of CO_2 seems to allow for even more rapid growth.

If it were the Sodium ion that is causing a difference then

Wine + Iron + H_2O_2 + Salt ($NaCl$) → Should give just as much a reaction as baking soda. I doubt it.

Notice the bleach reactions that are taking place also.

MMS I & MMS II are causing major reactions of growth also.

Vitamin C appears to be inhibiting the growth??

Case with + Baking Soda!

Wine + $FeSO_4$ + H_2O_2 + MMS II + TWC + (Antiox + Vite) → ???

!!! Theory is the OH° radical in presence of CO_2 allows for the most rapid growth.

Now lets compare the bleaches to H_2O_2

We know that

$Fe^{+2} + H_2O_2 \rightarrow Fe^{+3} + OH^- + HO^\circ$

now, MMSII is:

$Ca(ClO)_2$.

and we know what happens when this reacts with HCl

$HCl \rightarrow H^+ + Cl^-$

$Ca(ClO)_2 + 4HCl \rightarrow CaCl_2 + 2H_2O + 2Cl_2$

Now we know that Chlorine is a strong oxidizer!

Looking @ the results, it is appearing that the answer may lie with

maybe this alone ← $CuSO_4$

Baking Soda

Anti Oxidants

} or a Combination of the three.

Let's make up a solution of 0.5M $CuSO_4$.

Try: White + Culture (TWC) + H_2O_2 + MMSII + $CuSO_4$ + $NaHCO_3$ + Vit C + Anti Ox → ?

Let's prepare a .5M $CuSO_4$.
Our large eyedropper bottles are 60ml

If it was pure $CuSO_4$ we have

$(.5)\ \frac{159.61\text{gms}}{1000\text{ml}} = \frac{x}{60\text{ml}}$ $\quad x = 4.79\text{gms}$.

But it is not pure $CuSO_4$. It is $CuSO_4 \cdot 5H_2O$

so

$(.5)\ \frac{246.68\text{gm}}{1000\text{ml}} = \frac{x}{60}$ $\quad x = 7.40\text{gms}$

prepares a .5M solution of $CuSO_4 \cdot 5H_2O$

Too Strong. It needs to be 0.1 M solution

$= 1/5(7.40) = 1.48\text{gms}$ Done.

It appears that the culture grows very well with
1. White Wine 30ml
2. Iron Sulfate (5 drops)
3. H_2O_2 (2 drops)
4. Baking Soda (a pinch)
5. The transferred culture.
6. MMS II (a pinch)

So that which grows to most, we try to stop.

So we start w/ "MAX"

White Wine
Iron Sulfate 5 drops
H_2O_2 2 drops
Baking Soda (pinch)
MMSII (pinch)
Tle Transfered Culture

To Stop we add
(Cu) $CuSO_4$ (2 drop .1M)
(VC) Vit C. (pinch)
(AO) Anti Oxidants (2 drops diluted)

Need a set w/ [antidote?]

Transf. + Culture: White + $FeSO_4$ + H_2O_2 + Bak Soda + MMSII + $CuSO_4$ = ???
(Fe^{+2}) (OH) (CO_2) (Cl)

* Max growth appears to be with the hydroxyl radical in the presence of acid + CO_2.

You obviously need to explain what the Cu^{+2} is doing.

So Proposal coming up is 1. Baking Soda
2. $CuSO_4$ (or supplement)
3. Vitamin C
4. Antioxidants

exactly what you are taking

First of all, we need to know what MMS II is doing.

We have $Fe^{+2} + H_2O_2 \rightarrow Fe^{+3} + OH^- + HO^\circ$

We know baking soda is:

$NaHCO_3 + H_2O \rightarrow CO_2 + H_2O + Na^{+1} + OH^-$

Now what is baking soda doing in acid?

$NaHCO_3 + HCl \rightarrow CO_2 + H_2O + NaHCO_3\ NaCl$

(Water + Salt + CO_2)

Now what about MMS II?

We have already established w/ much work:

$Ca(ClO)_2 + 4HCl \rightarrow CaCl_2 + 2H_2O + (2Cl_2)$ another oxidizer.

now what about

$Ca(ClO)_2 + H_2O \rightarrow$

Well, we found something very interesting:

$Ca(ClO)_2 + H_2CO_3 \rightarrow CaCO_3 + 2HClO$ (hypochlorous acid)

(H_2CO_3: carbonic acid in water)

← Notice this. Baking soda will produce this.

And furthermore:

$2HClO +$ "organic matter" $\rightarrow 2HCl + O_2$

(what does this mean)

and

$2HCl + CaCO_3 \rightarrow CaCl_2 + H_2O + CO_2$

So which is it for us? Choose water for now.

We Have

$Fe^{+2} + H_2O_2 \rightarrow Fe^{+3} + OH^- + HO$

$NaHCO_3 + H_2O \rightarrow Na^+ + OH^- + CO_2 + H_2O$

and $Ca(ClO)_2 + H_2O \rightarrow CaCl_2 + 2H_2O + 2Cl_2$
(4 HCl in the stomach)

This leads to:

$Fe^{+2} + H_2O_2 + NaHCO_3 + Ca(ClO)_2 + 4HCl + H_2O \rightarrow$

$H_2O + Fe^{+3} + OH^- + HO + Na^+ + OH^- + CO_2 + CaCl_2 + Cl_2$

not enough of an alkalizer

alkalies

~~oxidizer~~ hydroxyl free radical

alkalies

salt

oxidize anyone oxidize in the body.

OUR PH here is still acidic 4.0-4.2 !!

This is the summary

This result says that the presence of the hydroxyl free radical (very damaging) in the presence of an ~~alkali~~ acid environment leads to explosive growth.

The presence of the organism in an acid environment leads to sustained growth.

The only solution is to alkalize the body (stops phase 1) and to kill the free radical (hydroxyl) with the appropriate antioxidant.

New set up
~~with Le+~~

It may be that the oxidation is a while but more important than your concern about CO_2 presence. I don't think right now that CO_2 is the problem, I think it is O_2
But this is still uncertain.

Guess what:

Max + Vit C + Antioxidant is working.

Max + Vit C + Antioxidant + Copper ~~is~~ is not working.

This is not true.

Max + Vit C + AO + Copper IS working.

Re Summary.

Now we know that

1. Fenton's reaction is important because it produces the OH° radical.

2. Acidic pH is important to growth. We know that an alkali solution holds it in suspension but does not kill it.

3. We know that CO_2 in addition to the OH° radical appears to be further promotional in growth.

4. We know that all oxidizers appear to be a detrimental influence, that is, they increase growth. This includes peroxide & bleaches of any kind. (MMS I, MMS II, bleach).

5. Antioxidants, esp. Vit C may be having a positive effect.

Observation:

This is the critical Statement

Given that a hydroxyl free radical exists within an acid environment (iron & peroxide will produce this free radical - Fenton reaction) then growth will increase rapidly within the presence of oxidizers.

a Potential Defense is:

Another Critical Statement.

Baking Soda
Copper Sulfate Solution (dosage to be determined)
Vit C
Antioxidants.

The hydroxyl radical can be produced with iron and peroxide alone, nothing else is needed.
This reaction is easily produced & tested for the existence of Fe^{+3} with the use of NaOH

Morgellons: A Discovery and a Proposal.

MMSII
$CuSO_4$ –

What is the Chemistry of the Solution?

We are seeing some results from

MAX + $CuSO_4$ + Vit C + Antioxidants,

The question now is can you remove anything which follows MAX.

To review, MAX is

White Wine – 30 ml
$FeSO_4$ 5 drops
H_2O_2 2 drops
$NaHCO_3$ ("pinch")
MMSII ("pinch") ($Ca(ClO)_2$)
the transferred culture

Defense Set:

Cu $CuSO_4$ (1 drop .1 M)
VC Vit C
AO Anti Oxidants (2 drops diluted)

Combinations

– Cu, VC, AO
Cu, VC
VC, AO
Cu, AO
Cu
VC
AO

This set must be tested.

Cu
Vite

Metabolism is Oxidation.
Example for glucose

$C_6H_{12}O_6 + 6O_2 \rightarrow 6CO_2 + 6H_2O$ This yields energy

Metabolic energy derives from processes of oxidation and reduction

White ⇒ Culture → $FeSO_4$
$+ H_2O_2 \Rightarrow$

$FeSO_4 + H_2O_2 \rightarrow$ Fenton's Reaction

$Fe^{+3} + OH^-$

Hydroxyl radical

HO OH

HO° OH°

Of the two solutions that show promise with Cu, AO & VC the pH is 4.5 & 4.7

The max solution by itself is 4.2

The pH of the wine is. 4.1

This indicates an increase in alkalinity.

When max has added to it Cu, AO & VC. The pH goes to 4.5

1. Key / Interlock release lever.
2. Wiggle the auto shifter.
3. all the way to the left

Mix:
White Wine
FeSO4 (5 drops)
H_2O_2 (2 drops)
Transferred Culture

Ferrous +2
Ferric +3

Now we have MAX:

	Antidotes
White Wine	Berry
FeSO4	Vit C
H_2O_2	Glycerin
Bak Soda	MSM (OMSO)
MMS	

"Lipid peroxidation" - The oxidative degradation of lipids. Free radicals steal electrons from the lipids in cell membranes, resulting in cell damage.

Hemoglobin contains iron. Hemoglobin generates Off. Off in presence of bacterial forms generate massive growth.

The blood's red color is due to iron ions in hemoglobin. Hemoglobin contains iron.

An "ester" is a organic compound (usually organic) by common usage
formed by reaction between alcohols & acids.

So what is an ester salt?

Sodium acetate is formed with acetic acid + sodium carbonate
or sodium bicarbonate
or sodium hydroxide.

Sodium acetate can be used to form an "ester"
with an alkyl halide such as bromoethane.

Aspirin, ~~aceto~~ & novocaine are esters.
So is malathion.

ATP is a phosphate ester.
Fats & oils are tri-esters.

Acetate is a salt ~~(or ester) of~~ acetic acid.

This is it.

An ester and an ester salt must not be the same thing. An ester salt is a salt from an organic acid.

Esters & salts have completely different properties.

Sodium acetate from acetic acid + sodium carbonate
or bicarbonate
or hydroxide

Sodium citrate from citric acid + sodium carbonate.

Min
10 citrate
5 glycerin booster
VC II

Min
10 acetate
5 glycerin booster
VC II

Min
5 citrate booster
5 glycerin

Min
5 acetate booster
5 glycerin

Min
5 citrate

Min
5 acetate
soaked up & added glycerin

Acetate	Acetate	Acetate
Glycerin	Glycerin	Glycerin
VCII	VCII	VCII
	AO	AO
		$CuSO_4$

Citric	Citric	Citric
Glycerin	Glycerin	Glycerin
VCII	VCII	VCII
	AO	AO
		$CuSO_4$

Under alka seltzers on Wikipedia you have learned that alka seltze produces sodium Citrate. This makes sense.
You are making it with lemon juice + sodium carbonate. Sodium bicarbonate also works.

The reaction is given:

Baking Soda

$C_6H_8O_7 + 3NaHCO_3 \rightarrow 3H_2O + 3CO_2 + Na_3C_6H_5O_7$

citric acid — baking soda — water — carbon dioxide — sodium citrate

Washing Soda

$2C_6H_8O_7 + 3Na_2CO_3 \rightarrow 3H_2O + 3CO_2 + 2Na_3C_6H_5O_7$

citric acid — washing soda — water — carbon dioxide — sodium citrate

You effective ingredients are

1. Sodium Citrate
   (Lemon Juice + Baking Soda)
2. Glycerine
3. Vitamin C.
4. If you add vinegar, you might be making sodium acetate. Seems less effective.
5. Copper shows no real sign.

So focus ingredients are

1. Lemon Juice
2. Baking Soda
3. Glycerin
4. Vitamin C

$CuSO_4$? (fitoment)

+ Alkaline Diet

Sodium Carbonate is Na_2CO_3
Sodium Bicarbonate is $NaHCO_3$

Oxidizing agents gain electrons

Oxidation is the loss of electrons

an oxidizer releases oxygen

An oxidizer causes a loss of electrons in another molecule or atom

oxidizers are often (but not always a source for oxygen)
dangerous around fuel.

It is time to start measuring Vit C powder
or absorbic acid powder by volume.

Need a small container that fills a large container.

Tle ½ teaspoon weighs 6.46 gms

a level teaspoon of Vit C III = 7.95 gms

50 of my small spoonfuls = ½ level teaspoon = 2.46ml

Therefore 7.95
- 6.46
= 1.49 gm = 1490 mg / 50 scoops = 29.8 mg / scoop

≈ 30 mg per scoop. Each vitamin C pill is 500 mg
So three tablets should make ½ tsp.

Min is:
1. White Wine
2. FeSO4 (5 drops)
3 H_2O_2 2 drops
4. Transferred Culture

To this we will add:
1. 30 mg Vit C III
2. Glycerine 2 drops
3. Sodium Citrate 5 drops

Spectrophotometers

found it

.5MU = 5 Angstroms

So 1 MU = 10 Angstrom

1 Angstrom = $1E^{-10}$ meters

nanometer = $1E^{-9}$ meters. visible light = 400-700nm

So 1MU = 10 · 1E-9m = 1E-8 m

So Beckman DB measures from 205 to 770 MU

So it measures from 205 · $1E^{-8}$ = 2.1E-6 m
to 770 $1E^{-8}$ = 7.7E-6 m
= 2100 nm to 7700 nm

vs 400 nm to 700 nm

So it is measuring a longer wavelength a a higher frequency.

But there is a problem:
UV light has a shorter wavelength
from 10 to 400 nm
Infrared is from 750nm to 1E6nm (1mm)

Region most useful for identifying organic compounds is 2500 to 16,000 nm
And this equals a frequency range of 1.9E^{13} to 1.2E^{14}Hz

The reciprocal Centimeter is the no. of ~~wave~~ wave cycles in 1 cm
Wavelength units are in microns instead of nanometers for the same reason.

~~20 H · C = 500 cm^{-1}~~

~~so C = 25~~
need the formula to convert from wavenumber (cm^{-1}) to wavelength in microns

$$\text{Wavenumber} = \frac{1}{\text{wavelength}}$$

$$\frac{1}{\text{wavenumber}} \cdot 1E7 = \text{nanometers}$$

so $\text{nanometers} = \frac{1E7}{\text{wavenumber}}$.

$$\text{Wavenumber} = \frac{1E7}{\text{nanometers}}$$

@ a wavenumber of 1500 = 6700 nm.
Exactly what the Beckman DB 8 can measure

Dosage Levels:

Take a human @ 70kg.

Assume Vit Ascorbic Acid @ 1000 mg/day

We are using 30 mg.

$\frac{30\text{ mg}}{29\text{ gms Wine}} = \frac{X}{70E3\text{ gms}}$ $X = 72,400$ mg

Equivalent Ascorbic acid over 3 months
3 days to 3 months.

$\frac{30\text{ mg}}{30\text{ gms}}$ $\frac{1000\text{ mg/kg}}{1E3\text{ gms}}$

Glycerol

$\frac{0.1\text{ ml Glycerol}}{30\text{ gms}} = \frac{X\ 233\text{ gms}}{70E3\text{ gms}} = \frac{3.3\text{ gms}/}{1E3\text{ gms}}$

$X = 233$ gms

12.6 gms / kg =

Glycerine density 1.261 gm/cm³

$\frac{.1261\text{ gms}}{30\text{ gms}} = \frac{X}{1000}$ $X = 4.2$ gms

A low means it absorbs.

Trial 2: 03/15/10 A high means it transmits.

Min
30 ml Wine
5 drops $FeSO_4$
2 drops H_2O_2

Antioxidants
5 drops Sodium Citrate
& 1 drop Glycerine
30 mg Ascorbic Acid

Spectrometry

Glass & water 318 & 740

1. White Wine has a peak ~ 684
low ~ 365 - 364
low 264

2. Blood : HbO_2

	Ref:	meas
high	514	514-515
high	564	563
low	540	543
low	578	578

3. Culture in Lye:
422 - 423 Low
260 Low

4. White Wine w/ Culture
805 - 804 low
382 low
~~255~~ 260 low (DNA?)

2 Glass & Water only
~~Peak~~. High @ 595
Two waves and two clear pegs the meter.
It may be the UV setting can be used
but it is too unsteady. Warm up? Pegs the meter.

Water & Wine:

High 601
Low 586
High 410
Low 372
High 320 glass?
Low 282

Wine & Wine Pegs the meter again.

Wine + Culture in Wine
32nd Low @ 450. Very Sharp Peaks
100 no High @ 330
45 00 ~~32 00~~ Low @ ~~256~~ 258 (DNA?)

Wine + Culture

760	72.8
740	72.0
720	71.0
700	69.6
680	68.0
660	66.5
640	64.5
620	62.1
600	59.7
580	56.9
560	53.0
540	49.0
520	44.8
500	40.2
480	37.5
460	35.0
→ 480	35.0
440	35.2
420	37.0
400	40.7
380	48.3
360	68.5
340	95.3
→ 330	100.0
320	97.2
300	71.1
280	50.6
→ 260	47.8
240	48.1
220	50.3

Every organic (live) substance that I have measured has a peak absorbance @ 260nm.

Culture / Wine
Avocado / Water
Cucumber / Water
Banana / Water
Aloe Vera / Water

and the 260/280 ratio is varying from 1.4 to 2.0

But be very very careful!

You put in Bleach / Water

and you also had a local high of absorbance @ 260nm.

You cannot trust the results until you get quartz cuvettes!

6AX5 Warm & Bright (to Hot)
85A2 Bright but not warm
6EM5 Dim & ~~very slightly~~ Moderately Warm
12AX7 Dim & very slightly Warm
12BH7 Moderately Bright & Warm to Hot
6973 Moderately Bright & Warm

6AX5	5.00
6SA2	10.00
6EM5	6.00
6973	$11.10 matched pair $19
12BH7	$19 (A) $
12AX7	$15-~~30~~ 25 - ~~$25~~ $15

Wine Culture 1" Cuvettes:

800	51.3		420	40.1
780	51.3		400	40.5
760	51.3	404	~~402~~	40.3
740	51.3	408	404	40.5
720	51.3	~~412~~	~~406~~	40.7
700	51.2	436		40.8
680	~~49.9~~ 50.9	432		40.8
660	50.5	428		40.7
640	50.0	~~426~~		
620	49.8	424		40.8
600	49.3	420		40.8
580	48.9	416		40.8
560	48.0	412		40.8
540	46.9	408		40.9
520	45.6	404		41.0
500	44.1	400		41.3
480	42.8	390		42
460	41.7	380		43.9
440	40.8	360		50.2
		350		52.8
		340		56.7
		300		56.2
		280		50.3

04128

Water Solvent

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(1) Water	(2) Culture (in cuvette in cuvette) % Trans			−1.1
317	100	460	69.1 =	68
340	92	470	68.9	67.8
300	90	480	68.5	67.4
420	72	490	68.4	67.3
460	67.8	500	69	67.9
500	67.5	510	69.5	68.4
540	71	520	70.2	69.1
580	76.8	530	71.0	69.9
620	85	540	72.1	71.0
660	86.8			
700	84.5			
740	82.5			
780	76.3			
820				
860	74.5			

Low Trans (Max Absorbance) @ ~ 490 nm

04/28

No culture here.

	(1) Water	&	(3) Wine
	310		100
	320		99
??	360		57.1
	400		73.8
	440		80.2
	480		82
	520		84.9
	560		89.8
	600		95.4
	640		96.8
	680		94.8
	720		91
	760		87
	800		81.5

	(3) Wine	+	(4) in Wine Culture
	310		80
	320		78
??	360		44
	400		55.2
	440		62.5
	480		66.8
	520		72
	560		78.3
	600		84
	640		86.2
	680		86.5
	720		85.5
	760		83
	800		79

Very Strong absorbance around 360 nm

We notice it is Completly different from the culture & water.

Not sure how to interpret this yet.

Notice we have a low again ~ 360.

What does the sharp absorbance @ 360 mean Lee?

Conditions of a solvent are

1. The "substance" must be soluble
2. Lower limit of transparency must be accomodate
3. Water & alcohol are good solvents.

What does it mean when the absorption varies according to solvent?
You will get a signature but how do you interpret it????

Is blood "soluble" in water? Our spectrum came out exact.

What does it mean to have such a sharp peak?
What is your real objective here?
1. A unique signature
2. Identification of a signature
3. Determination of resonant frequencies.

Wine + Culture

④	① Water	③ Wine
~~68.1~~	310	100
65.5	320	99
33.5	360	60.5
37.5	400	~~56.2~~ 58.7
41.9	440	56.5
44 →	480	55
47.8	520	56.2
52.5	560	61.5
58	600	70
60.7	640	76.8
61.7	660	76.2
61.7	720	75.2
60.5	760	73
58.3	800	68.2

⑤	① Water	③ Wine
320	320	99
325	330	90.3
330	340	79
335	350	67.8
340	360	59
345	370	55.4 ← Low
350	380	56
	390	58
	400	59.5
	410	60
	420	59.8
	430	59.2
	440	58.5
	450	58
	460	57.7
	470	57.3
Low →	480	57.2
	490	57.2
	500	57.7
	510	58
	520	59

Notice w/ culture added to the wine it shifts the low from 370 to 360. This does not actually look to be significant.

Then we have a peak at 700 vs 640. So indeed there may be some type of shift Comm. from the introvert—

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(1) Ethanol	(5) Ethanol + Culture
316	71
320	70
360	50
400	46.6
→ 440	46.8
480	46.5
520	49.9
560	55.5
600	61
640	63.2
680	64
720	64
760	62.9
800	60.3

360	50
370	46.4
380	47.6
390	47
400	46.7
405	46.4
410	46.2
415	46
420	46
→ 425	46
430	46
435	46
440	46
445	46.2
450	46.3
455	46.5
460	46.6
465	47.1
470	47.4
475	47.6
480	48.5

Increasingly it does look like we have max absorbance about 428 nm.

This is a trial in ethanol.
We also have the same results with wine as the standard.
This is now two different tests.

We are ready to start some frequency work.

Projected Resonant Frequency —

428 nm = 428E-9m

$\lambda \cdot w = 3EBm$ $\quad \lambda = \frac{3EBm/sec}{428E-9m}$

So $\frac{7.0093E14}{2^n} \leq 1E6Hz$ $\quad \lambda = 7.0093E14$ Hz

n=30

$\lambda = 652796$ Hz $\quad \cong$ 650K

n=31 326398

n=32 163199

n=33 81599

Try to get a light @ Radio Shack

428 nm

Estimated error in fundamental frequency.

Lets look @ harmonics of the light freq @ 420 nm.

$\lambda \cdot f = c$ now harmonics are usualy given in terms of a frequency

mean wavelength is high fundamental low no.
lower 1st harmonic higher
lower 2nd harmonic etc higher

We have a ~~freq~~ λ of 420 nm.

but you are lucky in alcohol & water.

$f = \frac{c}{\lambda} = \frac{3E0 \text{ m/sec}}{420E-9 \text{ m}} = 7.00943E14 \text{ Hz}$

now the idea is that this could be a n^{th} harmonic.

so we are seeking $\frac{7.00943E14 \text{ Hz}}{2^n} \leq 1E6 \text{ Hz}$

so

$2^n \cong \frac{7.00943E14}{1E6}$

$2 \cong \left(\frac{7.00943E14}{1E6}\right)^{1/n}$ $n \geq 30$ or greater.

Hz

$\frac{7.00943E14}{2^{30}} = (652804)$ This is suitable.

Now, what is the error in this frequency?

$y = \frac{X}{2^n}$ we want an error in y with respect to an error in X

$\frac{dy}{dx} = \frac{1}{2^n} \Delta X$

Now we need to know what is Δx?

$f = \frac{c}{\lambda}$ $\quad \frac{df}{d\lambda} = (-c)\lambda^{-2} = \frac{-c}{\lambda^2}$

$f = c \cdot \lambda^{-1}$ $\quad$ so $\Delta f = \frac{-c}{\lambda^2} \Delta\lambda$

let $\lambda = 428\text{nm}$ $\Delta\lambda = 10\text{nm}$

so $\Delta f = \frac{-3E8\ \text{m/sec}}{(428E-9)^2} \cdot 10E-9\ \text{m} = 1.6377E13$

so $\frac{df}{dx} = \frac{1.6377E13}{2^{30}} = 15252$ This is quite small.

This means expected error is +/- 15k Hz

ie 668056 Hz to 637552 Hz

= .67 MHz to .64 MHz

Most probable value = .65 MHz.

But there is something very interesting going on. The speed of light in water & glass and alcohol is not the same as in a vacuum.

But notice our resonant freq. was not determined in a vacuum. It was determined in alcohol & glass.

Water = 1.33

Ethyl Alcohol = 1.36

Glass = 1.6

So in test tube should be (about $\frac{(10)1.36 + 1.6}{11}$)

≈ 1.38

It is pretty close to this for human tissue also. But Charlene DNA patent has it at 2.83!!!

So she has freq in air divided by 2.83 to get human tissue.

But our frequency is determined in water, alcohol or glass. We estimate refractive indx for our work is 1.38

$f_{\text{alternate}} = \frac{C}{\lambda} \cdot \frac{1}{RI}$ where f is in the alternate medium.

~~$\lambda = \frac{C}{f \cdot RI}$~~ =

But what if we measure in the alternate medium

$f_{\text{vacuum}} = \frac{C}{\lambda} \cdot RI$ where f is in a vacuum

so $f_{\text{vacuum}} = \frac{3E8}{428E-9m}(1.38) = 9.53\,E14$

and $\lambda = \frac{C}{f} = 315nm$

$f_{\text{tissue}} = \frac{3E8}{315E-9\,(2.83)}$

$= 3.365\,E14$

$\frac{3.365E14}{230} = 313390\,Hz = .313\,MHz$

In tissue, not wire.

	(1) Ethanol	4 NaxCitrate	(2) Ethanol + Citrate	(3) Eth + Lye Sol'n	
340				95.6	
360				83.4	
380				73.3	
400				66.9	
420				62.1	
440				59	
460				58.1	← Low
480				58.8	
500				61.2	
520				64.2	
540				68.9	
560				75	
580				81.8	
600				87.7	
620				92.2	
640				96.9	High 96.9
660				97	←
680				96	
700				93	
720				88.1	
740				83.3	
760				76.5	
780				69.2	
800				61.3	

First frequency estimate

RI

For now assume Index of Refraction = 2.0

$$f = \frac{C}{\lambda \cdot RI \cdot 2^n} = \frac{3E8}{428E-9\ (2)} = 3.5E14$$

Now assume

OK $\frac{f}{2^n} \cong 1000\,Hz$ $\frac{3.5E14}{2^{39}} = 636\,Hz$

$f = 1000 \cdot 2^{\frac{1}{n}}$

$f^n = 1000 \cdot 2$

$n \cdot \ln(f) = \ln(1000 \cdot 2)$

$n = \frac{\ln(1000 \cdot 2)}{\ln(f)} =$

$6x^{-3} = -36x^{-9}$

Now $f = \frac{C}{\lambda \cdot RI \cdot 2^n} = \frac{C}{\lambda \cdot RI} \cdot 2^{-n}$

$$\frac{df}{dn} = -n \cdot \frac{C}{\lambda \cdot RI} \cdot 2^{-(n-1)}$$

$$\Delta f = -n \cdot \frac{C}{\lambda \cdot RI} \cdot 2^{-39.40} \Delta n \qquad \Delta n = 1$$

NO. $\Delta f = -12431$

Dark Culture form in Ethanol

	350	58.5	23.5	350 370	21.2
	360	51	21.2	352 372	2
	380	42.1	19.3	374	
	400	38.2	20.2	376	
	420	36.2	22.2	378	
→	440	35.8	25	380	
	460	36.2	28	382	
	480	38		384	
	500	40		386	
	520	43		388	
	540	47.1		390	
	560	57.2		392	
	580	58		394	
	600	63		396	
	620	61.2		398	
	640	72		400	
	660	74.7			
→	680	75.4			
	700	75.0			
	720	73.0			
	740	70.5			
	760	67			
	780	62.6			
	800	51.3			

Low is @ 384 when culture is dissolved in ethanol for a period of time. ranges up to 440 when solution is fresh.

384
+ 27
411 works good as an average

1. What we have accomplished & to verify that
2. Candidates
3. anecdotal Side
4. Outline of Research

Page 50

1. Lab Side
2. Personal Health
3. your Health

Papers:
1. Confirmation Independent
2. Reactive frequency Identified

1. Antioxidants
2. pH
3. Copper Sulphate
4. Light - frequency - absorption

1. Positive inhibition of early (bacterial) growth w/ antioxidants (glycerin, ascorbic acid, Na citrate) paper
   there is slight nagging need for repetition
2. pH

Topic
Outline of Future Research

Early Growth Repetition
1. Original sequence: transfer from red to white
   1. Transfer Trials
   2. Dental Direct
   3. Filament from Culture
      Iron & Peroxide Enhancement
2.

05/16

Reference Early Red Wine means:

1. White Wine
2. Early Stage growth transfer from red wine to white wine
4. $FeSO_4$ & H_2O_2 enhancement

Absorbance of Culture In Ethanol Alcohol

346 High
560 Low
710 - 720 High
~ 860 Low

Blood Absorption Spectrum vs Wave

	Absorbance					
340	38		548	34	880	64
360	38		552	33.7	900	59.3
380	39		556	33.2	920	57.5
400	70	Lo →	560	32		
~~384~~	54		570	33.5		
384	46	Hi →	580	35		
388	48		590	31.5		
392	54.5		584	33		
396	62.5	Lo	600	30.3		
400	71.8		620	31.5		
404	83		640	36		
408	94.5		660	40		
Hi → 412	95.7		680	43.5		
416	95.5		700	45.8		
~~Hi~~ 420	95.5		720	47.3		
424	85		740	48.3		
428	70		760	49.5		
432	54.5		780	56		
440	45		800	61.8		
460	33		820	71.5		
480	31		840	81		
Lo → 500	30.5		860	76		
520	30.6		844	81		
540	34		848	81		
560	31.5		852	80.5		
524	32.3		856	79		
528	32.3	Hi →	836	81		
532	32.7		832	81		
536	33		828	81		
540	34		824	81		
Hi → 544	34		820	79.5		

Some notes on the spectrometers.

The new mete seems more reliable & sensitive than the first meter.

On the new meter:

1. Absorbance scale seems the best to use by far.
2. If the solution is more concentrated you get a highe reading.
3. If the knob range is set to 0-1A it is more sensitive than 0-2A.
4. Leave the double beam switch alone! Leave on double beam
5. Ref knob can make minor adjustments in the needle,
6. The zero suppression knob works great! If you turn it counterclockwise in 1A mode, the needle can be adjusted to the right. The numbers on the suppression dial will decrease counterclockwise. The reverse is true for clockwise.

You can get very good results with this instrument if you are willing to adjust sensitivities.

Early Culture

Strong peak @ 804 & 352 nm
Low @ about 650 nm
This is in 5 drops of Lye in a test tube split in half between the two samples, a reasonable concentrated solution.
Suppression is set ~ 2 for the 650 nm range.

In ~~Water~~ Alcohol (Ethanol)
Peak is @ 340
Low is @ 500
and we don't have the 804 peak.
This indicates lye is a better solution.

Lye repeated - definitely superior.
High @ 804
Low @ ~ 500
High @ 360 this time.
You can see that it is dissolved in lye. but not alcohol.

The filament cultured in lye
We have a high @ 342 ~ 342
We have a low about 690
We do not have a definite high @ 804
Indicates it may not be fully dissolved.

Filament Cellulose in Lye

310	30.2	800	44
320	59	820	46
330	72	840	46.5
334	75	850	46.3
338	75.8	860	46.3
340	76.3	880	46.2
344	76.3		
348	763		
352	76.2		
360	~~76.3~~ 75.5		
380	68.5		
400	60.3		
420	52.5		
440	47.2		
460	~~480~~ 41.3		
480	37.3		
500	34.4		
520	30.2		
540	25.3		
560	20.3		
580	17		
600	15		
620	12.5		
640	10.5		
660	9		
680	7.5		
700	~~7.8~~ 6.8		
720	7		
740	10		
760	20.5		
780	35		

Early Culture in Lyre

310	~~16~~
320	~~46~~ 17.5 16
330	46
334	55.8
338	63.7
340	68.5
344	78.3
348	86.5
350	90.5
352	94
360	95.5
380	95
400	73.5
420	52
440	37.7
460	27.5
480	22
500	17.8
520	14.5
540	11.9
560	8
580	6.8
600	4.7
620	4.7
640	5
660	7
680	9
700	11.5
720	15
740	21.8
760	31.8
780	42
800	50
820	50.3
840	49.8
850	46.8
~~850~~	~~48~~
860	48
880	46

Blood in water

320	0
330	9.5
340	17.5
350	21.8
360	23
370	24
380	29
390	40
400	59
410	81
420	76
404	69.3
408	77
412	82.5
416	83
430	41
450	14.8
470	8
490	7
500	7
510	6.8
520	6.8
530	7
540	7.5
550	7
560	6
570	6
580	6.1
590	4.1

600	2
610	2
620	2.2
630	4
640	7.2
650	9
660	11.2
680	15
700	19
720	22.5
740	25
760	26
780	33
800	47
820	62.7
840	64.5
850	54.7
860	49.8
880	40
900	36

Volume of Oxygen Experiments

$\%O_2$ = Constant water level reading / tube length mm

Assumes length of tube is proportional to volume
(not exactly from curvature.
It is true for the graduated cylinders.

10 cm graduated cylinder filled w/ water.
Water weighs 16.63 gms.
Temp of water is 27.7°C.
Steel wool ball weighs .63 gms

1440: 15

~~1440~~ .5 min 10 mm 1453
~~1453~~ 10 min 18 mm 1458
1503

Steel wool weighs
7.9 gm/ml
We have .63 gms
$\frac{.63 \text{ gms}}{7.9 \text{ gm}} = .08 \text{ ml}$

Sugar:

$\frac{1.8 \text{ ml}}{16.63 \text{ ml} - .08 \text{ ml}} = .109 = 10.9\%$

1503 15 min $\frac{2.5 \text{ ml}}{16.63 - .08} = 15.1\%$

1508 $\frac{2.6 \text{ ml}}{16.63 - .08} = 15.7\%$

1513 2.5 ml

Final No is 2.6 ml ! $= 16\%$

BaO easily absorbs moisture and is used as a dessicant.

5:43:15 Ø
5:48:15 .7ml
5:52:15 .8ml

Mass of Steel Wool
15 ~ 1/3 of original
= .03 ml.
→ 16.60 ml now

---

5 Ø.9 mm
10 1.1

Steel .06 gms

Steel Wool 1.35 gms. $\frac{1.35}{7.9} = .17$ ml

5m 1.7ml
10m 1.7ml
15m
20m

147 ml = 147 gms

16.6 gms / ml

8.9 times

.633 m (9) = 5.13 gms steel wool

Big Container
Oxy ~ 6.5 gms Steel wool

5 min 20 cm

2.0 (10 ml) = 11.4 ml.
1.75

1.75 cm = 10 ml
5

9 gms 6.9 ml = .82 ml
7.9 gms

11.4 / 147 - .82 = 7.8% Oxygen

10 ml - 2.0 cm

2.0 / 10 = 1 / 5

Should be 4.9 cm high

% 5 cm = 30 ml

5 cm / 30 ml = 1 cm / X ml

X =

% O_2 = (# cm × 6) / 147 - .61 ml

Current wgt 4.82 gm Steel wool = .61 ml

Final Reading 5.8 cm
07/16/10
= 24%

5 cm is

Blood in Water Page 61

Hemoglobin Absorbance 02/26/11

	Absorbance	% Transmission
220	.465	34
230	.482	33.2
240	.49	32.6
250	.494	32.3
260	.495	32.2
270	.495	32.3
280	.495	32.2
290	.495	32.2
300	.474	33.7
310	.445	36.0
320	.447	35.9
330	.51	31
340	.60	25.3
350	.698	20.5
360	.78	17
370	.85	14.5
380	.95	11.2
390	1.1	8.5
400	1.24	5.5
410	1.43	4
420	1.44	3.5
430	1.19	7
440	.91	12.4
450	.72	19.1
460	.59	26
470	.51	32.5
480	.462	34.6
490	.455, .445	36
500	.445, .441	36
510	.458	35.2
520	.46	34.8

Success achieved. High Correlation.

λ	Absorbance	% T
~~510~~ 530	.46	34.8
540	.457	35.7
550	.435	36.9
560	.413	39
570	.39	41
580	.362	43.8
590	.345	.463
600	.308	49.5
610	.282	52
620	.261	55
630	.238	58
640	.221	60.4
650	.201	63
660	.185	65.3
670	.17	67.9
680	.156	70
690	.142	72.3
700	.127	74.9
710	.116	76.9
720	.109	78
730	.108	78
740	.109	78
750	.112	78.4
760	.116	76.8
770	.121	75.8
780	.127	75
790	.139	72.8
800	.145	71.6
810	.152	70.9
820	.127	75

Very good results against reference graph.

$CuSO_4$ in H_2SO_4

	Absorbance
200	.09
220	.13
240	.148
260	.153
280	.155
300	.122
320	.025
340	.065
360	.085
380	.096
400	.106
420	.116
440	.123
460	.131
480	.135
500	.137
520	.140
540	.143
560	.148
580	.155
600	.171
620	.196
640	.236
660	.287
680	.34
700	.395
720	.44
740	.45
760	.426
780	.40
800	.378

Now we must go after cable in test tubes by itself

200	.176		
220	.199		
240	.212	250	.201
260	.209		
280	.18		
300	.062		
320	.042		
340	.116		
360	.18		
380	.223		
400	.259		
420	.285		
440	.302		
460	.295		
480	.27		
500	.232		
520	.202		
540 540	.176		
580 560	.156		
600 580	.136		
620 600	.122		
640 620	.112		
660 640	.101		
660	.092		
680	.085		
700	.078		
720	.073	740	.068
760	.064		
780	.061		
800	.060		

EPA Filament in NaOH

200	.195		510	.202	
210	.202		520	.195	
220	.22		530	.184	
230	.224		540	.176	
240	.232		550	.169	
250	~~.243~~		560	~~.161~~ .165	
260	~~.243~~	?	570	.166	
270	.228		580	.159	
280	.228		590	.153	
~~300~~ 290	.221		600	.154	
300	.176		610	.146	
310	.136		620	.141	
320	.127		630	.136	
330	.18		640	.132	
340	.24		650	.130	
350	.292		660	.125	
360	.321		670	.121	680 .121
370	.323		700	.115	690 .119
380	.331		710	.116	
390	.332		720	.115	
400	.325		730	.115	
410	.316		740	.115	
420	.31		750	.115	
430	.302		760	.12	
440	.292		770	.116	
450	.262		780	.125	
460	.252		790	.135	
470	.242		800	.142	
480	.228				
~~490~~	.221				
500	~~.22~~ .212				

We have absorbance @ 260 = DNA?
transmittance @ 320
absorbance @ 390
transmittance 720-760

Now we work w/ culture spectrum,

Dried culture, Mortar & Pestle, NaOH & heated, Filtered, Same Color as EPA

Culture in NAOH

200	.325	510	.32
210	.342	520	.305
220	.354	530	.29
230	.353	540	.279
240	.371	550	.268
250	.372	560	.257
260	.372	570	.248
270	.371	580	.24
280	.362	590	.233
290	.345	600	.228
300	.292	610	.222
310	.264	620	.218
320	.298	630	.214
330	.372	640	.210
340	.465	650	.206
350	~~.579~~ .56	660	.202
360	~~.616~~ .62	670	.20
370	~~.685~~ .662	680	.197
380	~~.685~~ .670	690	.195
390	~~.675~~ .65	700	.194
400	~~.609~~ .619	710	.191
410	~~.59~~ .58	720	~~.189~~ .189
420	.543	730	.187
430	.514	740	.186
440	.48	750	.186
450	.452	760	.185
460	.426	770	.184
470	.402	780	.183
480	.38	790	.183
490	.36	800	.183
500	.338		

1. NaOH ← Character Description
2. Food Color
3. Dental Samples

NaOH - NaOH

Node: Analogous peak to H2O - H2O reference (440-450 nm) gets shifted to 490 in NaOH

No coincidence w/ filament samples either EPA or culture.

200		
220		
240		
260		
280		
300		
320		off scale low reached +- 320
340		
360		
380		steep climb up in absorbance
400		
420		
440		
460		
480	488	First Peak
500	488	2nd Peak
520	490	3rd Peak
540		
560		
580		slow steady decline in absorbance
600		
620		
640		
660		
680		Low Point
700		
720		rises
740		
760		
780		
800		
804		High Point

Live Dental Sample 2-27-11

200	.320
210	.370
220	.408
230	.435
240	.454
250	.461
260	.465
270	.465
280	.468
290	.462
300	.406
310	.331
320	.318
330	.368
340	.455
350	.537
360	.602
370	.640
380	.641
390	.625
400	.605
410	.590
420	.579
430	.570
440	.560
450	.555
460	.542
470	.538
480	.538
490	.539
500	.539
510	.523
520	.498
530	.458
540	.~~428~~ .418
550	.378
560	.342
570	.314
580	.287
590	.262
600	.237
610	.218
620	.202
630	.185
640	.168
650	.151
660	.136
670	.122
680	.113
690	.106
700	.103
710	.104
720	.115
730	.130
740	.155
750	.189
760	.232
770	.289
780	.348
790	.410
800	.433

2/27/11

Color Control Test - Blue

200	.	
220		
240		
260		
280		
300	.248	
320	.143	
340	.076	
360	.042	
380	.093	
400	.203	
420	.270	
440	.067	
460	.072	
480	.164	
500	.270	
520	.320	
540	.360	
560	.428	
580	.792	
600	1.600	
620	2.200	Est
640	2.200	Est
660	.660	
680	.120	
700	0.00	
720	.004	
740	.056	
760	.150	
780	.280	
800	.406	

Time to Start Drawing More Pictures.

What do we know:

1. Appears to feed off of iron
2. " " " " Calcium
3. Alkali & antioxidants help control
4. What is happening w/ this Cough syrup?
5. Explodes in presence of hydroxyl radical
6. $CuSO_4$ may have an influence

We may also need to develop a test quantitative for the presence of iron ~~KDAS~~ & their consumption. Calcium also?

What is a soluble form of calcium? (Calcium Citrate)
Calcium Carbonate w HCl

Test w/ Cough Syrup
$CuSO_4$

Eggshells + Lime Juice

Archaea
Can eat iron

Spectrum of Oral Sample Dweeks lake
Turns red

300	.174
320	.068
340	.161
360	.208
380	.218
400	.223
420	.227
440	.227
460	.222
480	.209
500	.218
520	.212
540	.201
560	.194
580	.186
600	.176
620	.173
640	.173
660	.174
680	.177
700	.182
720	.196
740	.223
760	.268
780	.326
800	.379
820	.312
.805	.390

A case of subtraction which worked very well!

Lets look @ Culture in Wine Vs Wine.

	Wine vs water.	Culture + Wine vs water
300	.008	.228
320	-.023	.185
340	.042	.265
360	.087	.36
380	.104	.382
400	.112	.365
420	.124	.352
440	.155	.365
460	.175	.382
480	.21	.398
500	.233	.380
520	.242	.363
540	.232	.332
560	.200	.209
580	.152	.229
600	.104	.175
620	.080 .082	.143
640	.078 .080	.180
660	.083	.126
680	.095	.133
700	.119	.153
720	.165	.194
740	.223	.252
760	.31	.342
780	.413	.452
800	.51	.556
810	.54	.590
820	.405	.462

This worked like a champ. Subtract the first from the second & scale it a few hours the culture by itself which matches again the EPA filaments

What we have is a bio spectrum here vs a chemical spectrum.

Our frequency generator goes from

10 Hz to 100 x 10,000 (= 1 MHz

We are @ 375nm =

Speed of light = 3E8 m/sc

$f \cdot \lambda = c$ $\quad f = \frac{c}{\lambda} = \frac{3E8\ m/sec}{375E-9\ m}$

$f = 8E14$ Hz

Refractive Index

= 800 terra Hz

= Velocity of light in a vacuum / velocity of light in medium $\quad$ THz

= 800E12 Hertz

Dielectric constant = (Complex Refractive Index)^2

in a non magnetic medium

Refractive index of water = ~~1.34~~ 1.333

White Wine & Red Wine ≅ 1.338

Human tissue may be about 1.533?

My guess is that we use the adjusted value instead of C

Using 1.330 speed of light in wire = $\frac{3E8}{1.330}$

= 224,215,247 m/sec

This is our new Constant for the speed of light

224,215,247 m/sec

so $f \cdot \lambda = 224215247$ m/sec

$f = \frac{224215247 \text{ m/sec}}{375E-9} = 5.97E14$ @ Hz

Now scale this down to 10 Hz to 1 MHz

2 —
2^2 —
2^{43} —
2^4 —
2^5 —
2^6
2^7 ½ 278
2^8 ½ 139
2^9 ½ 69.5
2^{10} ½ 34.75
17.37

$5.97E14 \times 2^{20} = 5.69E8$

$5.97E14 \times 2^{30} = 5.56E5$

= .556 MHz

= 556 KHz

$5.97E14 \times 2^{36} = 8687$ Hz

Lets look @ current estimate.

Signal generator = 5 watts

$P = I V^2$

$W = V \cdot I$

$I = \frac{W}{V} \approx \frac{5}{120} = .042\,A = 42\,mA$

$\frac{5}{0}\,\frac{W}{V} = .625 = 625\,mA$

seems right.

We have created a system of 212 uA @ 6.6V
How much power is this? (white wire disk)

In the red wire disk, we have no current - why?
OK, wire was not in the disk!
Now we have 212 uA

$P = 6(212E\text{-}6\,A) = .0063\,W = 1.3\,mW$

You have very little current, why is this?

Iron Culture

300	.122
320	.082
340	.150
360	.242
360	.333
400	.400
420	.43
440	.438
460	.432
480	.415
500	.395
520	.372
540	.337
560	.302
580	.263
600	.231
620	.212
640	.218
660	.222
680	.228
700	.235
720	.262
740	.308
760	.377
780	.470
800	.560
810	.578
820	.400

Subtracts —

What About			+Heat				+Heat
Water	+ NaOH	+ Culture	Water	+ NaOH	+ Culture	+ Blood	
320				300	.213		
340			320	310	.10		
360			340	~~320~~	.136		
380				360	.242		
400				380	.36		
420				400	.605		
440				420	.83		
460				440	.78		
480				460	.468		
500				480	.322		
520				500	.274		
540				520	.323		
560				540	.428		
580				560	.443		
600				580	.343		
620			600	~~600~~	.214		
640			620	~~680~~	.157		
660				640	.078		
680				660	.084		
700				680	.09		
720				700	.102		
740				720	.121		
760				740	.152		
780				760	.202		
800				780	.272		
810				800	.348		
820				810	.372		
				820	.232		

Let us look @ the spectrum of $FeSO_4$

300	.11
320	.021
340	.062
360	.098
380	.136
400	.15
420	.171
440	.186
460	.193
480	.193
500	~~.192~~ .196
520	.196
540	.166
560	.154
580	.144
600	.135
620	.136
640	.146
660	.152
680	.156
700	.166
720	.190
740	.231
760	.292
780	.37
800	.45
810	.47
820	.325

Lets lower the frequency

$\frac{5.97E14\,Hz}{2^{40}} = 543\,Hz$ 6.1V

Now a pulse wave

$\frac{5.97E14}{2^{38}} = 2172\,Hz$

Voltage is 6.7V

$y = \frac{C}{2^x}$

$y = C \cdot 2^{-x}$

$y' = -C \cdot 2^{-(x+1)}$

$\Delta y = -C \cdot 2^{-x+1} \Delta x$

$y = \frac{x}{2^n}$

$y = x \cdot 2^{-n}$

$y' = 2^{-n}$

$\Delta y = 2^{-n} \cdot \Delta x$

let x be off by 50 no
$= 2.965E14$

$\Delta y = 271\,Hz$

So
error range is

$543 - 271 = 272\,Hz$
$543 + 271 = 814\,Hz$

10^{n} error in frequency $= \pm 54\,Hz$
~~$= 214\,Hz$~~ to
$= 489\,Hz$
to $597\,Hz$

Working our problem backwards assuming a Rod material at 4Hz we get a refractive index of 1.421

Water is 1.333
Red Wine & White Wine ≅ 1.338
Human tissue may? be ≈ 1.533? (2002) (1)
We do seem to be in range—

power is 2^{47}

(2) Another source gets 1.382 (2005)
on bovine muscle tissue—

biophotonics - Variable behavior of light w/in the human body—

(3)
1.371 muscle
1.379 liver
1.352 pancreas
1.382 dermis. (2010)

Weighted average = $\frac{2(1.38)+1.533}{3} = 1.43$

"Intriguing Prospect"

ELF ↔ KHz → Light waves

Here is to thirty.
Best estimate of refractive index is approximately
1.382
1.371
1.352
1.382
1.379
$\bar{x} = 1.373$

Next. $\frac{3EB \text{ speed of light}}{1.373}$

$= 218499636$ m/sec in human body

body $\cong 2.185EB$ in the body approximate.

~~now $f \cdot \lambda = C$~~ (Air is 1.0008)

~~or~~

In Wine or water, the nO is:

wine/water $\frac{3EB}{1.338} \cong 2.242\ EB$

So now we have $f \cdot \lambda = C^*$
wavelength is fixed as we determined it in air.
so we have $f = \frac{C^*}{\lambda}$

water/wine $f_w = \frac{2.242EB}{375E-9} = 5.98E14$

body f_b ~~4~~

$f_b = \frac{2.185EB}{375E-9} = 5.83E14$

Now we look @ multiples.

In wine, we used a factor of 2^{40}

This leads to $\frac{5.98E14}{240} = 544\ Hz$

We have measured increase in growth at this frequency in wine / water @ 1mW. Is this an accident, would it happen anyway?

If we were to continue to the 4Hz fundamental we would need. $\frac{5.98E14}{247} = 4.25\ Hz$

but this is in water.
In the body
we would have

$\frac{5.83E14}{240} = 530\ Hz$

and

$\frac{5.83E14}{247} = 4.14\ Hz$ 4% error

To give a sense of allowable error we used 420nm.

$\frac{2.242E8}{420E-9} = 5.34E14$

$\frac{5.34E14}{240} = 485\ Hz$ = 11% error
we used ~~485~~ 544 Hz

Separation of Components

We know

$A = A_1 + A_2 + A_3 + \ldots$

$A = \epsilon \cdot b \cdot c$ $\quad \epsilon = \frac{A}{bc}$ $\quad \epsilon = \frac{A}{b \cdot c}$

So

$A = \epsilon_1 b c_1 + \epsilon_2 b c_2 + \epsilon_3 b c_3 + \ldots$

b = path length
c = concentration
A = absorbance

b is the path length, fixed. or $A = \epsilon b \cdot c$

basically a set of linear equations.

What is
- A log ratio of reciprocal of Transmittance
- ϵ a coefficient that expresses a standardization of absorption vs concentration
- b path length
- c is concentration

A little more clear to use:

$A = A_1 + A_2 + A_3 + \ldots$

$A = \epsilon_1 l \cdot c_1 + \epsilon_2 \cdot l \cdot c_2 + \epsilon_3 l c_3 + \ldots$

Now in our case we have mostly unknowns.

Now if we know how many A_i's there are we can solve for any of the A_i's individually. This is what we have done.

Eg, Culture A = Medium + Nutrients + Growth Form*

& Growth Form* = Growth Form + Lyt + Heat

Hemoglobin* = Hemoglobin + Growth Form.

You should be able to determine ϵ for a known chemical composition yourself.
You know the concentration, you know the path length & you measure the absorbance.

So what if for the concentration of the culture you just assume a reference value & call it 1.?
You could therefore determine an ϵ for it based upon that "reference concentration".

Your extraction of hemoglobin is wonderful.

What you are after the most here is the molar absorptivity of the culture. How would you do this?

First, you have the culture grown in various mediums. You would need to dry it out & weigh it.

Next you would need to subtract the influence of the culture.

This medium might be
H_2O
$H_2O + FeSO_4 + H_2O_2$
$H_2O + FeSO_4$
Red Wine
White Wine.

But you will always have NaOH + heat added to the culture & this must be subtracted out also.

So we would have
NaOH + Culture + Heat + Medium

vs

NaOH + Heat + Medium

This would give us culture.

So the medium will need to be exactly defined.
If you don't know what it is you have a problem.

In our blood problem, we know that
blood now contains both blood and the growth form
(to varying degrees).

So what we did was subtract the culture
form to get the blood ie

NaOH + Culture + Blood vs NaOH + Culture.
Gives hemoglobin.
(Here it did not matter what the medium was,
it was all subtracted out)

NaOH + Culture + Medium vs NaOH + Medium
will give the culture.

So you have to know what the medium is to
get to culture properly.

Question is, do you have any way of purifying
the culture so that it has nothing else in it?

Sure, just take the medium out. No matter
what it is!!! Then you get to culture!

We actually have the instrument calibrated fairly well for midrange measurments. If we get ≤ 0 absorbance it just means it is so low that it does not matter.
Remember though to medium however we need to add a lye component.
How do we do this at the right concentration.

What we are doing is adding - call it 3 drops of lye and heating it, so this is what we need to add to our medium reference solution.

So take medium.
add 3-4 drops to water
add 3 drops lye
heat it - this becomes the reference solution.

Then add to culture.

Then subtract the reference.

That is the culture.

(add it to blood for example)
pbe

The lye component is not right.
What you are doing is taking 3 drops lye adding it to the culture & heating it.
Then you take 1 or two drops of that solution and dilute it in water. This is your solution for spectroscopic analysis. So the lye solution is highly diluted after it is heated.

So this is what you need to know do before you add it to the reference solution.

Bottle it:
Take Medium.
Add a few drops to water.
Add 3 drops lye
Heat it.

Take a few drops from that result & add it to water.
That becomes your reference solution.

Simpler approach:

water + Iron Sulfate + H_2O_2 + lye + Heat = Reference Solution.
(medium)

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Now add to culture

	1 drop Water	1 drop $FeSO_4$	1 drop H_2O_2	1 drop NaOH	1 drop Lye	Heat
300		.02		.303		
20		0		.24		
40		.03		.32		
60		.115		.46		
80		.175		.56		390 .~~48~~ .58
400		.19		.592		410 .~~58~~ .564
20		.194		.555		
40		.19		.50		
60		.174		.448		
80		.156		.402		
500		.138		.36		
20		.12		.319		
40		.104		.274		
60		.085		.24		
80		.065		.205		
600		.045		.175		
20		.042		.162		
40		.056		.162		
60		.076		.169		
80		.093		.~~18~~ .177		
700		.116		.192		
20		.152		.222		
40		.205		.264		
60		.278		.335		
80		.365		.42		
800		.452		.505		
810		.~~44~~78		.536		
820		.37		.37		

Now subtract them.

This method is successful. It is just a matter of refinement now. The culture has a very sharp peak at anywhere from 375 to 400 nm (There is some uncertainty here).

We also now have blood by itself.

We now add them together.

You have succeeded.

Now, you can make this grow very easily. How can you stop it?

Conception of Iron is a huge problem!!
You can tie up the oxy hemoglobin in your blood. You need to stop it from eating iron in the blood. This is a huge problem.

M. Conference [redacted]

What lib. Made of?
difficulty w/ labs
Control sample w/ cotton
2nd control sample - blind -

C-O-S, 35K electron -
Commercial extrusion -
what exactly was the sample? Thought it was M. sample?

natural & environmental ? why ?

Cotton thread dyed w/ indigo, dye?
Not saying why or how he is selecting samples

Next me: Cellulose, Cotton
Not saying criteria for selection of samples

15 min in.

Down sample

Rodent hair

Are these all control?

Human hair w/ cellulose fibers surrounding.
hair brushed after shampooing -
"
A mixture of a lot of stuff

Example of 600 microns

~20 min

" "

Now into biological with no internal structure
a "fungal fiber"

Debris attached to a human hair.
oily secretions He is going on + on w/this.

Why is he doing this?
FTIR, mass mass element analysis,
microscopy - .

25 min 1. No elucidation yet
2. Analyses expensive

What next:

So now he is planning to go back + focus SG4 + SG7
on 2 of previous samples
and apparently ask for new samples. - No, he has new
" "

Picking the tapered sample that looks like it was cut.
This is bizarre.

30 min Now he is involved w/ new samples! Crystalline
2. a filament, but we still do not know
where it comes from. "A candidate fiber".

35 min - 40 min -
DNA

Disability Specialist

Nicolaus, MD

Dr. ~~Nicholas~~ (Garsten)
from Austria
"Morgellons in Europe) - Tick ~~Borne~~ Borne Diseases.

" Disease" increasing in Europe
Problem to get epidem. data.
Will rely on personal experiences.
" Definitely using disease".

Holistic Therapy Concept
1. Med History & traditional lab testings
2. Checklist for Co-infections
3. Risk Assessment for Inflammation, M. "patients"

Lab tests: 70% Lyme disease or tick borne.
Lyme & Borrelia testing.
these tests done before testing
Also testing for conventional bacterial co-infections

He does mention Chlamydia "Coinfection" @ 85th level & regards it as high.

And now he checks for viral infections.

Blood test to exclude autoimmune diseases

EKG testing.

Problem here is that we are mixing everything into the kitchen sink rather than a specific focus on a specific organism.

"Treatment"
Anti-parasites (Antibiotics)
Diet (Major vitamin + mineral deficiencies)
Detoxification

Pain
Exercise
Stress
Mental Coaching & Social Support
All appears to be very beneficial to generalized health improvement.

Diet:
Resveratrol
Alkalizers
Assume Vitamins -

I see a generalized approach to a little of everything to a "disease"
Essentially
No support or knowledge of the 'disease'.
4 doctors in Germany or Europe?

[redacted]

Nicolaus (Cont)

Goals "

Morsellous

1. Eliminate parasites" bacterial infection.
2. Minimize side effects
3. Stabilization

References heavily oriented toward lyme disease.

Charles Holman Foundation.

[redacted]

Individual Case Study

[redacted]

What part of the body?

Can it be collected?

MUSIC

[redacted]

Diagnostic Criteria:
1. biting, stinging
2. subcutaneous fibers
3. 10% do not have lesions

Then She does identify filaments as crucial & Characteristic of the Condition.

She says not visible by naked eye.

Hair loss
Fibers in teeth & crumbling of teeth
She is correct
She mentions calcium leaching
bone loss suspected - She is Right

Sold Mount on Head

Digital Microscope on skin-session.

* "None of us know what we are ~~talking~~ talking about here".. how telling —

Some useful exterior observations presented here.

* "Everything we say here is pure guesswork".

Dr Greg Smith: (Pediatrician) on track

Elizabeth Rasmussen pltd

Good Common sense observations & Contradiction w/ DOP diagnosis.

DNA presentation:

Nuclear DNA vs Mitochondrial DNA (hair, etc) nails

No presentation of any results just the technology.

Now [redacted], now going back over previous work using DNA methods.

" "

Shotgun DNA Cloning/Sequencing

He ~~was~~ a receives an accidental single skin analyses and ends up w/ Candida & Staph & Keratin shows up.

* Chimp DNA, from my understanding, IS 99% +/- the same as human ~~anyday~~ anyway.

Humor does not apply here →

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Now he is talking about

"

creating cultures"

bacteria,

fungi

Wynne himself says OSHA sick reports everywhere except Antarctica

Waste Water

Blood work
Stem vs Blood -
Magnification — how to
Filaments - External vs Internal, What do you analyze?
Hardiness - Ability to survive
Living vs Artificial
Intelligence
IRON
Elimination from the body vs acceptance of existence

[redacted]

Oxidation - need for Oxygen
Environmental Source

Alk/Php -

#4

1. Iron
2. mitigation
3. where storage

Back to our work:

We know that we have a 2 component system

$A = A_1 + A_2$

$\text{Total} = \overset{\text{Pure}}{\text{Blood}} + \text{Culture}$

But blood is contaminated, culture is contaminated.
To get culture we realize!
$\text{Culture}^* = \text{Culture} + \text{Medium}$
So
$\text{Culture} = \text{Culture}^* - \text{Medium}$
so now we have

$\text{Total} = \text{Pure Blood} + (\text{Culture}^* - \text{Medium})$
Now Pure Blood is difficult to get pure
so we can use a theoretical model spectrum
or create our own by subtracting influences
But theoretical at same resolution as our
instrument is fine.
So

$A\ \text{Total} = \text{Pure Blood}_{\text{Theoretical Spectrum @ same resolution}} + (\text{Culture}^* - \text{medium})$

$\text{Culture}^* = \text{Culture} + \text{Medium}$ (~~not measured~~)
↑ Measurable
Medium is also measurable.
(Culture is difficult determinable.)

Deviation from True Blood is the issue
What would be the extreme?

04/25/11

We have blood H_2O_2 quite well now (ie a theoretical model)
We also have impure blood & we see some shift of the main peak and a new peak @ about 398.

Now let's try to separate out the culture influence

Culture + Lye +heat + $FeSO_4$ + H_2O_2 is what we are working with.

Next we filter this.

Next we add 6 drops to H_2O & this becomes our sample.

Prep Prop of blank.

1 drop $FeSO_4$
1 drop H_2O_2
4 drops NaOH
+Heat

Take 6 drops of this & add to water & this becomes our blank

None of this IS sterile but it IS a start.

The Concentration was not sufficient. This experiment was a failure.

04/25/11

Proposals for Spectrometry:

You now have a modern fractional spectrophotometer. It is working like an absolute champ.

You have already demonstrated one of the most important applications that could ever be hoped for:

Identification of the "Morgellons" condition within the blood of an individual.

What can you do with this instrument?
Essentially establish a unique signature for anything you can get into solution that has color.

What are other ways that you can use this instrument?

1. Severity of symptoms can now be correlated w/ spectral analysis of the blood.
2. Various regimes of improvement or strategies can be established and the progress monitored in a convenient timely & objective manner. (eg diet), detox, etc. alkalinity, antioxidants
3. Concentration of the blood solutions will need to be examined as it relates to the shape (magnitude) of the spectral plot.
4. Various sample types & forms can be examined for their consistency with a known spectral signature of the condition.

or culture forms

5. Now a unknown sample types can be examined if they can be brought into solution.

6. Concentrations of the culture can be calibrated with a known mass (dried) in a given solvent.

7. The influence of the medium of growth upon the spectral plot can be examined for the purpose of determination of purity of the semple.

8. Effects of nutrients upon growth can now be examined from a spectral perspective, ie is iron or calcium absorbed directly into the organism?

9. The rate of growth can now be examined as to what point (form of growth) sufficiently establishes the spectral signature.

10. Comparisons w/ known biological species, such as Chlamydia can now be made (again, if they can be brought into solution).

11. Other forms of tissue can be examined for the existence of the organism (if brought into solution) - eg hair, saliva, etc.

Whatever you do, it does need color in the solution to work.

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We now know that the culture growth w/in only $FeSO_4 + H_2O_2$ is the same as the mature filament growth.
Also the anomalous blue light form is the same.
Everything is the same from a spectral point of view.

Now we can go to work on Concentration levels.
Weight of crucible is 49.57 gms

With moist sample wgt is 51.40 gms
You now dry the sample by heating the crucible until it is dry
Weight of dried sample in crucible is: ~~50.00~~ gms
No, Crucible needs to cool. Ok it settles @ 50.01 gms
So wgt is 50.01
49.57
.50 gms exactly. very good.

Now we will heat 100 ml of water.
We will add 10 drops of lye with the Pasteur eyedropper.
We will heat it to boiling & let it boil (simmer for 5 minutes) 1 minute only.
We will filter it & this will be the stock solution Concentration 1.

You could have pulverized the culture after it was dry but I did not. Not pulverizing it, however, is more representative of what you actually do.

You have, however, broken it up into knife size pieces.

1234 is boiling. Boiled for 1 minute only.

3 millilites to be placed in each test tube.

lets dilute it by 1/3 instead of 1/2 each time.

1	3	
2	$3 - 1/3(3) = 2$	$2 / .0625 = 32$
3	$2 - 1/3(2) = 1.333$	$1.333 / .0625 = 21$
4	$1.333 - 1/3(1.333) = .89$	$.89 / .0625 = 14$
5	$.89 - 1/3(.89) = .59$	$.59 / .0625 = 9$

Count drops to measure better:

$$\frac{64 \text{ drops}}{4 \text{ ml}} \quad \frac{1 \text{ drop}}{X} \qquad X = .0625 \text{ ml}$$

measure @ 398 nm

Calibration (Concentration) Curve is Successful.

Concentration Levels Assumed:		Measured Absorbance
1	1	.848
.666	$1 - 1/3(1) = .667$	.762
	$.667 - 1/3(.667) = .444$	.538
	$.444 - 1/3(.444) = .296$	.288
	$.296 - 1/3(.296) = .197$	.163

The solution given is: (this term is the product of a & l)

$$\text{Absorbance} = .9159 * \text{Concentration} + \cancel{\emptyset}$$

So

$$\text{Concentration} \cong \frac{\text{Absorbance}}{.9159}$$

$\lambda = 398$

Example:

$\frac{.848}{.9159} = .93$ vs 1.0 Not too bad.

$\frac{.762}{.9159} = .83$ vs ~~.75~~ .67 Not too good

$\frac{.538}{.9159} = .59$ vs ~~.54~~ .44 Not great

$\frac{.288}{.9159} = .31$ vs ~~.29~~ .30 great

$\frac{.163}{.9159} = .18$ vs ~~.16~~ .20 great

So now we have a Concentration estimator

Concentration	$\simeq$	Absorbance
$\lambda = 398nm$		.9159

A Concentration of 1.0 means:

.50 gms dried culture = 500 mg
100 ml of water
10 drops (.62 ml) of 1.0 NaOH
Heated to boiling
Boiled 1 minute
Filtered Solution

So @ any point now if yu measure the absorbance of a culture solution (in lye) at 398 nm

Yu now have a good estimate of Concentration with

Concentration	$\simeq$	Absorbance
$\lambda = 398nm$		.9159

For a 3ml solution in a test tube a Concentration level of "1" means

$\frac{.50 gms}{100 ml} = \frac{X}{3 ml}$ $\quad X = .015 gms$

$= 15 mg$ of culture per 3 ml of solution

So in milligrams

"normal" units

Concentration in $\lambda = 398 nm$ $= \frac{Absorbance}{.9159}$

Choose an example:

Absorbance = .62

$\lambda = 398 nm$

$Concentation = \frac{.62}{.9159} = .68$

so a Concentation of .68 means

$.68 \left(\frac{5 mg}{ml}\right) = 3.4 mg/ml$

So Concentration $= \frac{Absorbance}{398} \left(\frac{5 mg}{ml}\right)$ $\frac{}{.9159}$

in mg/ml

or Concentration (mg/ml) = Absorbance * 5.46 $\lambda = 398 nm$

*

A Concentation of 1:

$\frac{.50 gms}{100 ml} = \frac{X}{1 ml}$

$X = .005 gms/ml$

$= 5 mg/ml$

We believe the actual amount now is 5/4

$= 1.25 mg/ml$

· This works.
From any graph you can now assess the concentration of the culture in the solution.

This portends a serious problem in the blood. The peak is very measurable!

You are seeing rise from 0.7 to 1.2

0.7 ≅ 3.8 mg per ml of blood?

1.2 ≅ 6.5 mg per ml of blood?

How much blood is in the body? 5.6 liters

~~10 pints~~ =

5.6 liters = 5600 ml.

5600 (6.5 mg) = 36 gms in the blood.

means 10 times as much as I put in the solution.
This is a lot!

04/28/11

Tonight you have some mixed results
There are some questions of interpretation.

In general the environmental samples are more difficult to break down & interpret than the culture filaments are.

Lye in water against lye in water is indeed Ø.
but
Lye in water heated is giving some change.
A = .23 to .17

But now when you add the
1. Bean sample
2. NY Filament Sample (on deck)
3. California Prison sample

You get essentially the same result.
Peak at about 358 nm.

Now when you get to the Magellons culture, the absorbance is much higher and the peak is 390 and a sharp drop immediately.

So we have environmental being the same, culture being the same within, but both sets are different.

04/30/11

You have now proven that the environmental sample is the same as the culture sample w/ the Spectronic 20D.

It is learned that it is much more difficult to break down the environmental filament VS the culture form. This really should not be a big sprise.

It is taking lye + Curling solution to have any impact - these are extreme in breaking down keratin a protein bonds.

It is ~~separ~~ difficult to concentrate the solution enough to rise above the noise but you have finally done so.

We have some solution in lye + Curling solution that we should now save for culture work.

The next thing that you learn is that the organism proliferates in a blood sample that has been in storage, idle for some time. This means if the body cannot keep the impact down it will eventually overwhelm the organism.

The spectrophotomer will detect the presence of the organism but it will do nothing to eliminate it.

We have a little problem. We put Iodine in and we get the same sharp drop from peak @ 396. Why?
This is not good. Does not make sense.

This is a problem. Same stead dropoff @ 396 nm. Why & how could this be?

Left: Current Sequence:

Water
Iodine
Yellow Food Dye
Lye
Curl Wire
Blood

Lye + Curling
Environment ~~Element~~
Blood
Culture + Whey + $FeSO_4$ + H_2O_2
Whey + $FeSO_4$ + H_2O_2
Blood

You had a little scare.

The glass test tubes are giving a false peak near ~ 396 nm
(So is cuvette, but it is much smaller)
It happened for both Iodine & yellow food color and you have no idea why. For some reason, the plastic cuvettes do not have this same problem.

Best work, or if any doubt, will be in plastic cuvettes.

Your work is still valid, however for the culture & blood. Effect is still to create a false peak and shift the main peak to the right.

You are not sure why Iodine & yellow food color are affected but if you get a strong peak @ 396 make sure you compare to a plastic cuvette.

Nevertheless you have proven your work & have a caution to watch out for.

Lets look at the Iodine suggestion:

Assume 20 ml of culture solution.
How much Iodine can the body take?
Why is regular Iodine toxic? vs Lugol's?
What is in Iodine antiseptic solution?

Antiseptic Iodine contains "povidone-iodine"

It is polyvinyl pyrrolidone

LD 50 = 8000 mg / kg = 8 gms / kg

Nausea, vomity & abdominal cramps
80 kg
LD human = 640 gms.
So assume you operate on 1/100 of LD 50 = 6.40 gms.
Now how long a time period would you like to extend this? 30 days =
= .213 gms = 213 mg per day to 30 days

Now what is the amount of Iodine in 1 ml of solution?
10% solution = .1 gm per ml.
For us, with a 0.5% solution, we have .005 gms / ml
= 5 mg / ml

Now if we add 5 drops into our culture
5 drops (~ .0625 ml / drop) = .3125 ml
so we would have .3125 ml (5 mg / ml) = 1.56 mg / ml

98-99% of people can take
10-200 mg a day without symptoms"

Donald Miller, cardiac solution
Aug 14 2006

In our work,

5 drops in our culture = 1.56 mg

Now if we were to have 20ml of solution
This would be equivalent to

$$\frac{1.56\text{ mg}}{20\text{ml}} \quad (20\text{gms}) \quad = \quad \frac{6240\text{ mg}}{80\text{E}3\text{ gms}} \quad \text{deadly!}$$

If you use 1 drop = 1248 mg
You would need to divide this by 10.

So you would need to use 1 drop
& dilute it by 10 drops to be in range.
$\approx (10).0625\text{ ml} = .62\text{ml}$ OK.

So you really need to take your solution
& divide it by 10.

.5% needs to go to .05% solution

.05(60)ml = 3 ~~mL~~ 3ml Iodine
+ 57ml water
60 ml

This is what you need to use.

So to use Betadine internally,

Take 10% solution
Dilute it by 200

$\frac{100\ \cancel{mg}}{4}\ \frac{0.1\ gm}{\frac{1\ ml}{200}} = \frac{.0005\ gms}{1\ ml\ (gm)} = \frac{X}{80E3\ gms}$

$X = 40\ gms$

$\frac{X}{1\ ml} = \frac{.100\ gm}{80E3}$ $X = .00000125\ gms$
$= .00125\ mg$

This is how much is allowed per ml.
We have 20 ml, so = .025 mg allowable
in culture per day.

We have a 0.05% solution = $(.0005)\cdot\frac{1\ gm}{ml} = .05\ \frac{mg}{ml}$

This means we can add:

$\frac{.025\ mg/\cancel{ml}}{.05\ mg/ml} = 0.5\ ml$ per day

$\frac{0.5}{.0625\ ml/drop} = 8$ drops per day

Which says to me you can use the 0.5% solution.

Again, but with Ø.5% solution:

$$\frac{.1 \text{ gms (allowable)}}{\text{BOE3 (human being)}} = \frac{X}{1 \text{ ml}}$$

(ml)
(gms)

X = .00125 mg/ml

We have 20 ml, so:

20(.00125 mg/ml) = .025 mg allowable in culture each day.

Now we have a Ø.5% solution mixed up

$$= \frac{.005}{1.} \frac{\text{gms}}{\text{ml}} \qquad X = \frac{.00025 \text{ gms}}{\text{ml}} / \text{ml}$$

~~to our~~ in our solution,

which equals Ø.25 mg/ml in our stock solution

but we are only allowed .025 mg in the culture each day.

So

$$\frac{.025 \text{ mg}}{.25 \text{ mg/ml}} = Ø.1 \text{ ml allowable in culture each day}$$

and, in terms of drops, this equals approximately 1 drop per day.

We have a good reference plot of H_2O_2 Conc.

We took log of molar absorption coefficient & it works very well

Now scale to match our max absorbance of 1.65

$$\frac{13.17}{11.6} = \frac{1.65}{1.93}$$

$$\frac{13.17}{11.6} = \frac{1.65x}{6.82}$$

$$x = \frac{11.6}{1.65} = \frac{7.03}{1.93}$$ Scale factor

$$\frac{13.11}{x} = 1.73 \qquad x = 7.61$$

We have a very encouraging result.

As a baseline, we take a spectrum of

(1) whey + $FeSO_4$ + H_2O_2

Next we take the culture & place it in this same solution & give it an opportunity to grow for 4 days.

(2) We end up heating & filtering both solutions to allow for the breakdown of the culture. (Notice we did not add NaOH, however)

When we take the spectrum of case #2, however, we get no difference from Case #1.

* This indicates that the culture is not growing.

It is time now to analyze the impact upon the blood.

We could assume model of

$A = A_1 + A_2$

or

$A = C_1 A_1 + C_2 A_2$

This could be a least squares solution.

$measured = C_1 \cdot Ref + C_2 \cdot Culture$

You have plenty of measurements.

If we would measure @ culture out to 1000 we have a better solution.

You could pick data at strategic points

$$\begin{bmatrix} Ref & Culture \\ & \end{bmatrix}_{n \times 2} \begin{bmatrix} C_1 \\ C_2 \end{bmatrix}_{2 \times 2} = \begin{matrix} Meas\ 1 \\ Meas\ 2 \end{matrix}_{n}$$

Culture Model

	Stock Ref	Culture	Meas	Δ	C_1 Ref + C_2 Culture = Meas Model	
* 340	1.521	.777	.695	-.21	.909	.94
* 368	1.495	.999	.963	-.13	1.092	1.124
* 397	1.630	1.04	1.741	.00	1.740	1.184
* 414	1.730	.891	1.65	-.03	1.68	1.723
* 426	1.668	.831	1.807	.00	1.803	1.85
* 506	1.301	.544	.413	-.15	.565	.59
* 541	1.430	.410	.832	.25	.584	.61
* 560	1.366	.352	.571	.05	.525	.55
✓ 576	1.436	.308	.839	.34	.499	.52
✓ 667	.749	.153	.111	-.14	.234	.21
✓ 686 ~~668~~	.737	.133	.106	-.13	.235	.24
✓ 700	.745	.12	.103	-.12	.225	.24
✓ 926	.935	.039	.081	-.11	.189	.20
1000	.911	.036	.079	-.10	.182	.20
✓ 816	.892	.062	.087	-.14	.222	.24
* 380	1.525	1.045	1.168	.03	1.136	1.17
* 400	1.642	.934	1.474	.41	1.062	1.10
* 360	1.506	.971	.891	-.17	1.07	1.10

$V + B\Delta = f$

$V = f - B\Delta$

This gives the weights of the influence of the culture.

$X = -.02$

$\sigma_{n-1} = .180$

$\widetilde{Meas} = .183 + .812$ ~~.107~~ (Ref) + ~~.844~~ (Culture)

This solution looks very good.

You made a mistake in some of the data entry.

Page 125

We have done a pretty good job.
We need more data between 520 & 600 nm

		Ref	Cult	Meas	Model	
✓1	500	1.307	.573	.428	.704	.73
✓2	510	1.302	.53	.41	.668	.69
✓3	520	1.326	.487	.464	.637	.66
✓4	530	1.392	.45	.605	.619	.64
✓5	540	1.430	.412	.83	.595	.61
✓6	550	1.402	.379	.687	.563	.58
✓7	560	1.366	.352	.571	.535	.55
8	570	1.460	.321	.726	.527	.54
9	580	1.422	.299	.746	.502	.51
10	590	1.258	.277	.313	.454	.46
11	600	1.061	.254	.172	.4	.41

Now 29 data pts:
New $\bar{X} = -.02$
$\sigma_{n-1} = .186$

This work is a complete success.

Current Model is $.181 + .854 \text{ Culture}$ (~~.185 Ref + .872 Culture~~) This term should be equal to concentration × path length.

$A = a_x C_x l + a_y C_y l$

$= Ax$

I found several mistakes in my data entry

Absorbance is proportional to Concentration * path length

You have proven, to a very good approximation,

that the influence of the organism upon the blood produces the spectral plot that you have measured upon affected blood.

You therefore have established a method of detection of the presence of the organism within the blood and the impact of the organism upon the blood.

Protocols & strategies ~~may then be developed~~ as a of mitigation of impact may therefore be measured objectively.

The model also tells us that in the visible light range that the organism has roughly 4 times the influence (log scale) in the absorbance of energy over that of hemoglobin.

Notice on hemoglobin there is a sharp rise in absorbance @ 400 nm. but notice in the culture there is an extremely sharp drop in the absorbance. Because of the weights of influence, notice the culture influence overwhelms the hemoglobin influence at that particular wavelength.

Let's go over the model and see if we can turn into an actual determination.

We have a model of the form:

Meas = .103 Reference + .812 Culture $\sigma_{n-1} = .186$
The theory is

$A_m = A_x + A_y$

$Am = a_x C_x l + a_y C_y l$ we meas @ 2 wavelengths λ_1 λ_2

$Am' = a'_x C_x l + a'_y C_y l$ where ' refers to an implicit wavelength

l is the path length, known
C is the concentration
a' is the absorption.

For our culture we have prepared a stock solution
Concentration (mg/l) = Absorbance * 5.46
$\lambda = 398$ nm

Now we need to go over units in book.

$\frac{g}{dm^3} = \frac{\text{grams}}{\text{liter}}$ $l = dm^3$

$l = dm^3 = 10^{-3} m^3$
$ml = cm^3 = 10^{-6} m^3$

Relationships

$A = acl$
$A = \epsilon C l$

a is absorbtivity
ϵ = molar absorbtivity (standardized)
C = Concentration
l = path length

Units:

1 dm = 10 cm

$1\ dm^3$ = one cubic decimeter = $1000\ cm^3$ (10 x 10 x 10)

So 1 liter = $1\ dm^3$

1 liter = 1 cubic decimeter

It is indeed an unusual unit but take it as it is.

I would just use liters or ml.

Ok, now that we understand the unit assume we are given

$$\frac{.40\ gm}{liter} = \frac{.40\ gm}{1000\ ml} = \frac{.4\ mg}{1\ ml} = .0004\ \frac{gms}{ml}$$

p130 Thomas Spectrometry

In comparison, our stock culture solution is 5mg/ml

The other example is $\frac{.17\ gm}{1000\ ml} = \frac{.17\ mg}{ml}$

So it is roughly a 2 to 1 concentration of the first compound to the second.

Now the absorbtivity is given (defined as)

$$\text{Absorbtivity } (a) = \frac{\text{Absorbance}}{\text{Concentration}}$$

units are $\frac{\text{liter}}{\text{~~liters~~} \cdot gm \cdot cm}$ Now, why?

This unit analysis was incorrect. Look ahead 5 pages +/-

$$\frac{.90}{.40} = \frac{\text{absorbance}}{\text{gm/l}} = \frac{\text{absorbance}}{1} \cdot \frac{l}{\text{gm}} = \frac{\text{absorbance} \cdot l}{\text{gm}}$$

$$= \frac{\text{absorbance}}{\text{gm}} \cdot dm^3$$

This means absorbance has units of $\frac{1}{cm}$ NO!

And we know that this is true, so now it is making sense

5. Absorbance units are $\frac{1}{cm}$

Absorptivity units are $\frac{l}{gms \cdot meters\ cm}$ or $\frac{ml}{mgs \cdot cm}$

Absorbance vs pathlength is a linear function. (logarithm function is incorporated within).

absorbitivity is $\frac{\text{absorbance}}{\text{concentration}}$ so it is essentially absorbance scaled by the concentration.

We also see that absorptivity is a function of wavelength so it is hardly a constant.

Next "molar absorptivity" is standardized even further by using a sample of concentration calculated in moles/liter

This is all looking good.

So to solve a 2 component solution we

1. Measure the absorbance of the two solutions in a pure form at a min of two frequencies.

2. We also measure the mixture @ the same two frequencies

3. We need to know the concentrations of each of the pure solutions.
From this we can get the absorbtivities (a) at the two frequencies.
by relation $\text{Absorbtivity} = \frac{\text{absorbance}}{\text{Concentration}}$

4. Now using the relation
$A = A_1 + A_2$
or $A = a_1 \cdot C_1 \cdot l_1 + a_2 C_2 l_2$
We are after the C's, or the concentration.

5. Our system of equations is:

@ λ_1 $(a_n C_n l)$ $A_1 = a_1 C_1 \cdot l + a_3 C_2 l$
@ λ_2 $A_2 = a_2 C_1 \cdot l + a_4 C_2 l$

Now the l terms cancel out.

So the Matrix form is

$V + B\Delta = f$

$$\begin{matrix} e^{\lambda_i} \\ \lambda_{i+1} \\ \lambda_{in} \end{matrix} \begin{bmatrix} a_{1\lambda_i} & a_{2\lambda_i} \\ \vdots & \vdots \end{bmatrix} \begin{bmatrix} C_1 \\ C_2 \end{bmatrix} = \begin{bmatrix} A_{M_i} \\ \vdots \\ 0 \end{bmatrix}$$

which is exactly what we did but we did not scale by concentrations.

Lets establish what the Concentrations actually are.

Red blood cells are about 1/3 hemoglobin. (gms/deciliter)

MCHC Mean cell hemoglobin Concentration
(Avg Concentration of hemoglobin in a given volume of blood)

32 to 36

Normal ranges humans is ~~2 to 3~~ ~~33.6~~ ~~pico~~ gms
~~pico~~ dl

a deciliter is 1/10th of a liter or 100 ml Ok

Now we know we are putty in about 3 drops in about 3 ml of water.

So roughly this is:

$\frac{44 \text{ drops}}{3 \text{ ml}} = \frac{1 \text{ drop}}{x}$ $x = .065$

44 drops = 3 ml

~~drop~~ 1 drop = .068 ml per drop. This is essentially same as before .0625

So avg is .065 ml/drop

So human blood has approximately

$\frac{34\ gms}{100\ ml}$ (seems very high) but this is what it is.

Now we are placing 3 drops in 3 ml of water, or 1 drop in 1 ml. of water. Our concentration is therefore:

$\frac{1}{100}$ $\frac{34\ gms}{100\ ml}$ (.65 ml) = .0221 gms

for 1 drop of blood.

We are placing this in 1 ml of water.

$\approx \frac{22\ mg}{ml}$ This is our approximate concentration. (So it is very heavy, since it is not

$=$

Now the concentration of our stock solution is:

$\frac{5\ mg}{ml}$ So now we know what we need.

/

Now the form of our equation was:

$A = .183\left(\text{Reference Hemoglobin Absorbance}\right) + .812\left(\text{Culture Absorbance}\right)$

But now we know from Thomas p 130 that we should have scaled our measured absorbance values by the concentration levels to get the absorptivity coefficients which is what we wanted. So we set up

ok

$A =$ Absorbance of Reference C_x + Absorbance of Culture C_y of mixture

and solved for C_x & C_y when we should have set up

$$A_{\text{mixture}} = \frac{\text{Absorbance of Reference}}{(22\ \text{mg/dl})} C_x + \frac{\text{Absorbance of Culture}}{5\ \text{mg/ml}} C_y$$

to get C_x & C_y. Let's try to fix

22 ∴ So I think C_x needs to be divided by 22 & C_y by 5

$C_x = \frac{.183}{22\ \text{mg/dl}} = .008$.

$\frac{.812}{5} = .162$?

$C_x = 4.015$ gms/ml

$C_y = 4.058$ gms/ml

This would have the hemoglobin concentration being way way way too low. !!!

These results are amazing.

This assumes the hemoglobin is at the concentration level it should be, ie 22 mg/ml.
But what if it were much less?
What if say it was only 11?

Then what would happen?
The current results suggest that the effects are fairly evenly balanced.

If we decrease the concentration of hemoglobin by 1/2, ie 11 mg instead of 22 it has the effect of causing

$C_x = 2.008$ It cuts it 1/2!
$C_y = 4.050$ holds the same

So it shifts the influence to the culture more.

Remember our solution only has 1 drop of blood per ml!!!

First of all, whole blood is about 1/3 hemoglobin.

You are estimating that you are using about 1 drop of blood per ml. This means you are getting about 1/3 (~~.065 ml~~) · 22 mg/ml = ~~1 mg~~ 7.3 mg/ml of hemoglobin per ml. is what I need to compare to, not 22 mg/ml.

Fact? Situation: Hemoglobin mass in human blood is ~ 22 mg/ml. and about 1/3 of human blood is red cells hemoglobin.

But the hemoglobin density in blood is fixed @ ~22 mg/ml

I happen to take about 1 drop of blood per ml in my solution. That I am measuring absorbance of. 1 drop ≅ .065 ml.

So my blood is diluted by a factor of approx $\frac{1+.065}{.065} = 16.4$

Therefore I would have to increase my concentration of the reference hemoglobin by 16.4 to get my measured values.

Confusion: I want to get the reference value at 22. Combine of my to set stock solution to get the measured values. How do I do this? Multiply by 120

Ok, ya now have a solution that is working.

fix this

Model of Measured Affected Spectrum $= 21.9 \frac{\text{mg}}{\text{ml}} \cdot$ Reference Hemoglobin

let this float

$+ 4.058 \frac{\text{mg}}{\text{ml}} \cdot$ Reference Culture.

And ya arrived at this by: ↘ we know Concentration of the Culture

This says the expected Concentration in the blood is roughly 80% of the stock solution.

Method was:

.50 gms dried culture
100 ml of water
10 drops 1.0 NaOH
Heated
Boiled 1 minute
Filtered.

This is true if is a reference

4 mg/ml is an estimate.

We have learned that there is ~ 5600 ml of blood in the body.

$5600 \text{ ml} (A) \cong 22.4 \text{ g mg/ml}$ ≅ 40 pellets

=

So even though you don't exactly know what it is, you do know the concentration of mass under the solution.

You can use this w/ the model you created to determine the concentration w/in the blood.

The net effect of this is that you have a method to estimate the amount of growth w/in the body in the blood.

How would you generalize this to any individual in the next problem.

Now it is time to look @ the problem from scratch.

Absorbance $= \frac{1}{Cm}$ (= 1/L)

$A_M = a_x C_x l + a_y C_y l$.

how does l actually cancel out?

You are diluting blood so it complicates matters. Lets take this problem from scratch and see what the layout is.

If you had straight blood and a culture mix, what would you do?

$A = A_1 + A_2$

Mixture = Blood + Culture

$A = \log \frac{I_0}{I} = A = abc$

A is the absorbance
a is the absorptivity (proportionality constant)
b is the cell length
C is the concentration

So A is proportional to product of concentration & cell path.
Now, what are units?

A is a number. I do not see that it has any units. Correct. It is unitless

Units of a: $a = \frac{A}{b \cdot c}$

A is a number
b is a distance (centimeters)
C is $\frac{gms}{liter}$

So a has units of $\frac{1}{\frac{\cancel{cm}\ 1}{\frac{gms \cdot \cancel{liter}\ cm}{liter}}} = \frac{liter}{gms \cdot cm}$

= This matches Vol 19 spectrometry book.

Assume Pure Blood for Now

So $A = \frac{\text{liter}}{\text{gm} \cdot \text{cm}^2} \cdot \text{cm} \cdot \frac{\text{gm/s}}{\text{liter}}$

Correct. A is dimensionless.
So I will prefer

$A = a \cdot C \cdot l$ and it all equates to a number.

so now

Mixture (number) $= a_1 C_1 \cdot l + a_2 C_2 l$

$a_1 C_1 \cdot l$: Blood Hemoglobin

$a_2 C_2 l$: Culture

Now we know the concentration of blood, at least in theory.
but we also have
$A_1 = A_1 + A_2$ if one of the quantities is known (as hemoglobin is) we can use this information

so

A (mixture) number we measure this. $= A_1$ known hemoglobin for a particular concentration. (Can be standardized) $+ a_2 C_2 l$

Reference! Culture:

$A = a_1 C_1 l + a_2 C_2 l$

A: always known

a_1: always known

C_1: this varies but we know it.

l: known, should be determinable

a_2: known

C_2: known

l: known

This is also conditional - specifically @ 390 nm
for our stock culture solution we
know some things.

We have $(a \cdot l) \cdot c$

A(Stock culture) = .9159 × Concentration Ratio + ∅

and we know what a concentration ratio of 1 means
what you are now allowed to do is take
a random dilution a concentration of the
solution & you know what the concentration
is by simply measuring the absorbance.

Now it must be measured, however at
the peak frequency of 390 nm.

This is a critical condition.
Now what do we really know about this
concentration?

What we do know from it is a
concentration in "mg/ml" that
has been developed from a
process. It is not an absolute
but it is a very important reference
solution.

A concentration of "1" means 5 mg/ml
to which the "process" has been applied.

Now back to our situation.

@398nm $A_{mixture}$ = reference hemoglobin + $a_2 C_x l$

$= a_1 C_1 l$

↓ We know this constant $= .9159$

We can measure this.

We could compute this product.

We can fix the Concentration ratio as 1. or we can also dilute it.

Couldn't we set up two equations? @ two different Concentrations of Stock solution?

@398:

$f_1 = a_1 \cdot C_x \cdot \phi.5\,cm + .9159$ (at Concentration Ratio = 1)

meas.

$f_2 = a_2 \cdot C_x \cdot \phi.5 + .450 = .5$

meas

We would be learning $a_1 \cdot C_x \neq a_2 \cdot C_x$ but now C_x by itself

Still a problem.

Lets create some kind of standard
Combination & see what it looks like.

What if we use

How about

Stock Solution + Blood

vs Stock Solution?

Start w/ 25 drops 5/55 Stock culture
= 25(.06 ml) = 1.5 ml Stock

add 1 plastic drop blood (barely)

#5L Culture
#6L Culture + Blood

I clearly get the same peak
structure as blood by itself.

This means the blood has lost.

So this did not help any.

Calibration of Eyedroppers

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Calibration of eyedroppers once and for all.

We did the plastic pipette dropper today

1 drop = .068 ml

44 drops / 3 ml 46 / 3 ml

X = .065

Glass Dropper (Perelandia)

52 drops / 3 ml = .058

Actually less than the other

We did this before and got .0625 X = .06

So

glass = .06 ml/drop

plastic = .065 ml/drop

$A = a_x C_x l + a_y C_y l$

Now thomas p130 is defining

absorptivity as $\frac{\text{Absorbance}}{\text{Concentration}}$ This is wrong. It is $\frac{\text{absorbance}}{\text{conc.} \cdot l}$

this would be

units of $\frac{1}{\frac{1}{\frac{\text{gms}}{\text{liter}}}} = \frac{\text{liter}}{\text{grams}}$

but we know this is not actually true!

because

absorptivity units are actually $\frac{\text{liter}}{\text{gms} \cdot \text{cm}}$

he is, neglecting the cm term.

So his equations do not seem to be set up correctly. Should be

271nm $.41 = \frac{.90}{.40 \cdot 1\text{cm}} \cdot 1\text{cm}\, C_x + \frac{.34}{.17 \cdot 1\text{cm}} \cdot 1\text{cm}\, C_y$

215nm

OK, so it does cancel and he is just leaving it out.

Now we have a better understandy of units.

We have a model:

$$M_{mixed} = .183 \cdot \underset{\text{Hemoglobin}}{\text{Reference}} + .812 * \text{Culture}$$

from $A = abc$ or $A = \varepsilon c l$

A is proportional to product of concentration * path length

so a should be $\frac{.183\ l}{gms \cdot \emptyset.5\,cm} \cdot C \cdot \emptyset.5\,cm$

so what this leads to is

that

$$M_{mixed} = .183 \cdot C_{reference\ hemoglobin} + .812\, C_{culture}$$

This says Standard hemoglobin is 22 mg/ml

So @ 398 nm

$$C_{culture} = \frac{M_{mixed} - .183(22\ mg/ml)}{.812}$$

$$= \frac{1.141 - .183(22)}{.812} =$$

The model is not working. Why?

You have a problem w/ your math model & you have chosen to negate it. It does not combine linearly You also had errors in your input data which was a very poor error.

1. Now the question is, how do they combine?
2. How can you determine concentration of each.

You need to think about this →

You can not just add them and take least squares solution essentially ended up looking like the culture.

What if you multiply?

a model of the form

$$\text{Mixture} = \text{Reference}^{a} \cdot \text{Culture}^{b}$$

$a \tilde{=} 1.1$
$b = .75$ is producing the best results.

Why?

y_1 Black = Hemoglobin
y_2 Red Dash = Culture
Dotted = Measured (2)

Black: $\exp(\frac{3}{4} * y_4) / 100$

Culture $\exp(2 * y_5) / 15$

$(y_4 \times y_5) 1.75$

y_4 $\exp(3 * \text{hemoglobin}) / 100$

y_5 $\exp(2 * \text{meas}) / 15$

$y_4 * y_5$

$C_1 \cong 3 \quad 3.2$
$C_2 \cong 2 \quad 3.2$
$a_1 \cong 100 \quad 125$
$a_2 \cong 5 \quad 15$

So model that works is

$$\left(\exp(C_1 \cdot y_1)/a_1\right) \cdot \left(\exp(C_2 \cdot y_2)/a_2\right)$$

$$\frac{e^{C_1 y_1} \cdot e^{C_2 y_2}}{a_1 a_2}$$

$$\left(3 * \frac{y_4 + y_5}{4}\right)$$

Best model is:

y_1 = hemoglobin
y_2 = culture

$$y_4 = \exp\frac{(C_1 \cdot y_1)}{a_1}$$

$$y_5 = \exp\frac{(C_2 \cdot y_2)}{a_2}$$

then $\frac{d_1 \cdot y_4 + d_2 y_5}{d_1 + d_2}$

so

$$\frac{\frac{d_1 e^{C_1 y_2}}{a_1} + \frac{d_2 e^{C_2 y_2}}{a_1}}{d_1 + d_2}$$

$$= \frac{d_1 e^{C_1 y_1} + d_2 e^{C_2 y_2}}{f}$$

but we see $C_1 \cong C_2$ so

$C_1 \cong 3.2$

$$\frac{d_1 e^{C_1 y_1} + d_2 e^{C_1 y_2}}{f}$$

$$\frac{3e^{2 \cdot x_1} + 4e^{2 \cdot x_2}}{f}$$

$$d\left(\frac{e^{C_1 q_1} + e^{C_2 q_2}}{d}\right)$$

the better model is:

$$\frac{\frac{d_1 e^{C_1 q_1}}{a_1} + \frac{d_2 e^{C_2 q_2}}{a_2}}{d_1 + d_2}$$

best estimates:

A	$C_1 = 5.2$	3.2
B	$C_2 = 3.2$	3.3
C	$d_1 = 3$	3
D	$d_2 = 1$	1
~~F~~ E	$a_1 = 125$	125
~~G~~ F	$a_2 = 15$	15

You now have a very good model form that combines the spectra.

I am not sure if it is generally known how this works, but you have solved this.

$\log A + \log B = ?$ $\log(ab)$
This does not work, at least directly.

So now your problem is, how would you arrive at Concentration.

The linear model assumes $A_M = A_1 + A_2$
~~In the theory assumes a linear form.~~
But clearly they are not acting together in a linear fashion.

Experiment w/ Crossing yellow & Blue
Food Dye

40 ml water, 1 drop yellow

	Left	Right
#1	Water	
#2	Yellow	
#3	Blue	
#4	Mixed	

Clearly the Mixed spectrum is not an average of the two components.

Experimental Combination does indeed look purple. Mixed equal quantities.

The model says
$A_M = A_1 + A_2$

$A_M = a_x C_x l + a_y C_y l$

So what the model is saying is that the Absorbance is a linear combination of Concentrations

NOT a linear Combinations of spectra, or absorbances.

This Changes the picture dramatically
Concentration is not the same thing as absorbance (or spectra).

$A_m = a_{xl} \cdot C_x + a_{yl} \cdot C_y$

340 $.319 = \frac{.292}{1.709}(.5)\, C_x + .0177\, C_y$

So

$$B \quad \Delta = f$$

V+	.292	.077	C_x	.379	340*
	1.709	.092	C_y	1.204	447
	.245	.243		.427	496
	.039	.373		.374	560
	.022	1.801		1.171	630.5
	.022	.011		.166	700

$C_x = .694$
$C_y = .636$

Works like a champ. A Perfect Solution!

So even though you dont know the actual concentration you know the relative concentration. You assume it should be equal to $\frac{1}{2}$ but it is not. but the work is perfect.

You have a perfect (essentially) solution to combining yellow and blue food dyes.

How to do this we need the reference spectrum for hemoglobin

and a spectrum for the culture.

$A_M = A_H + A_C$

or

$A_H = A_M - A_C$

A_M = AFFECTED BLOOD

A_C = CULTURE

or

$A_H = A_M + (-1) * A_C$

$A_H = a_x l \cdot C_x + (-1) a_y l C_y$

	May 07 (3) Affected		Apr-30 (2) Culture	(1) Red H
340	.631	+	~~.393~~ .541	1.523
397	1.689	+	1.026	1.628
401.5	1.219	+	.831	1.648
430	1.625	+	.826	1.631
510	.39	+	.549	1.302
541.5	.834	+	.441	1.430
576	.842	+	.346	1.436
599	.164	+	.3	1.08
700	.09	+	.178	.745

$C_x = -.096$

$C_y = -2.344$

This is a very poor fit. Why?

414	~~1.731~~ 1.498	−	.866	1.731
558	~~1.3~~ .526	−	.386	1.310
600	.093	−	.192	.739

better now

It is not as smooth as food dye but it still works

Moall Culture

$A_H + A_C = A_{affected}$

$C_x = \cancel{.075} -.13$ $\quad X = -.0294$ $\quad n = 12$

$C_g = \cancel{1.765}\ 1.055$ $\quad \sigma_n = .24$

Now try to get these on a spreadsheet — You have something here.

Take Affected - Culture:

$$Affected - 1.765\ Culture = -.075\ A_H$$

$$A_H(-.075) + 1.765(A_{Culture}) = A_{Affected}$$

$$A_H = \frac{A_{Affected} - 1.765\ A_{Culture}}{-.075}$$

Hem Culture Affected

$$-.075(1.523) + 1.765(.393) = .579$$

Hem Aff - Culture

$$-.075(1.523) = .579 - 1.765(.393)$$

$$1.523 \doteq \frac{.579 - 1.765(.393)}{-.075}$$

$$\text{Hemoglobin} = \frac{\text{Affected}}{.631 - 1.765(.541)}$$

Weak Blood & Strong Blood are Different!

Now we learn that weak blood and strong blood have very different spectrum.

Remember the blood is altered.

It is actually very different.

1. Look @ the peak of 448 w/ strong blood.
2. Notice the very sharp peaks @ ~540 ~575.

We have to start working w the concentration problem.

Let's try to get 3 solutions.
Peak of normal blood is 414
But strongest peak is 577 that is common to both hemoglobin & affected species

1.5 ml → 3 → 6 ml

We believe this is actually ~.006 ml/drop now

2.0
~~1.5~~ ml + 10 "drops blood"
blood

1 drops = estimated @ ~~.03~~ .02 ml 10 drops = ~~.3~~ .2 ml

2.0
= ~~1.5~~ + .3 ml = ~~1.8~~ ml 2.3

10 drops in 2 ml
5 drops in 4 ml

Approximate Concentration:

~ml	We have 4 solutions of 1 ml each (but double up in cuvette)	
.2	#1 10 drops in 2 ml	~70 mg
.1	2 5 in 2 ml	~35 mg
.05	3 2.5 in 2 ml	~17.5 mg
.025	4 1.25 in 2 ml	~9 mg

Ok, you have a good calibration curve to blood now @ 577 nm:

Absorbance $\tilde{=}$ .2451 * Concentration in drops/2ml
Now to equate this to drops per ml
It should be
or Absorbance $\tilde{=}$ .1225 * Concentration in drops/ml

Ok, we have good results here.

Now lets translate this to absolute amounts.
We are estimating each "drop" of blood $\tilde{=}$ .02 ml

$$\frac{34 \text{ gms hemoglobin}}{100 \text{ ml of blood}} = \frac{X}{.02 \text{ ml}} \qquad X \tilde{=} \frac{.007 \text{ gms}}{\text{per drop}} \tilde{=} \frac{7 \text{ mg}}{\text{drop}}$$

S.

Now lets change our absorbance equation.

We have

Absorbance $\approx$ .2451 * Concentration in drops / 2ml

but Concentration in in $\frac{mg}{}$ $\approx$ Drops * 7mg

So if something measures 2.5 absorbance
What does it mean?

$$\frac{\text{Concentration in drops}}{2\text{ml}} = \frac{\text{Absorbance}}{.2451}$$

Example $\frac{2.5}{.2451} = 10.2$ drops

$$\frac{\text{Concentration in mg}}{2\text{ml}} = \text{Concentration in drops} * 7$$

$$\frac{\text{Concentration in mg}}{\text{ml}} = \text{Concentration in drops} * 3.5$$

Absorbance $\approx$ 0.49 * Concentration in drops / ml

$$\text{Concentration in drops/ml} = \frac{\text{Absorbance}}{0.49}$$

$$\text{Concentration in } \frac{mg}{ml} = \frac{\text{Absorbance} * 7}{0.49}$$

or even more simply

$$\frac{\text{Concentration of Hemoglobin in mg}}{\text{ml}} \cong 14.3 * \text{Absorbance @ 511nm}$$

If you are off it is only because of the estimate of the blood drop as approximately .02 ml. I think it should be pretty good as approx 1/3 of an actual drop.

OK, this is useful. Now we need to go after the stock solution.

We are taking a stock solution of 30 ml & boiling it down to 10 ml. (.0625) Looks to me like we used 1 ml, not 2

ml	drops	Δ from 2 ml	Δ drops H_2O	Conc
2		0	0	~~.33~~ 3
1.33	32 21	~~.61~~	11	2.0
1.33 .89	21 14	~~.44~~ 1.11	18	1.33
.89 .59	14 9	1.41	23	.89
~~.59~~ .39	~~9 6~~	1.61		

We have a good solution

Absorbance = .5491 * Conc. @ 446.5 nm

Note

or

Concentration of Culture = $\frac{\text{Absorbance @ 446.5}}{.5491}$

You now have two good calibration curves:
One for hemoglobin & one for the culture:

Conc. of Hemoglobin in mg / ml = 14.3 * Absorbance @ 541nm

Conc. of stock solution = Absorbance @ 446.5 / .5491

You also see an entirely new peak in the culture @ sufficient concentration @ 446.5 nm

The additional peak @ 397 nm explains the variation in the hemoglobin spectrum @ sufficient concentration.

Now we need to learn how to combine the spectra again.

Somehow we need to subtract the culture from the affected blood and see how closely we get to hemoglobin.

Now lets determine our concentrations

1. Strong Blood Hemoglobin @ 577 $\quad A = 2.451$

$$Conc = 2.451 * 14.3 = \frac{35\ mg}{ml}$$

2. Culture @ 446.5 $\quad A = \frac{1,734}{1,491} = 3.25$

$$\& \quad 3.25\left(\frac{5\ mg}{ml}\right) \approx 16.2\ \frac{mg}{ml}$$

Next we know that

Affected Blood = Hemoglobin + Culture

Measured $\quad$ To be Determined. $\quad$ Measured

~~.799 $\quad A \times C \times l \quad +$~~

~~$+ =$ Affected $-$ Culture~~

You just took a straight difference.

It shows that the problem is even worse than anticipated.

The difference plot shows that, at sufficient concentration the organism completely overwhelms the hemoglobin spectrum for approx 340 to 500 nm. You also have two marker peaks, not 1. You only need to have blood at a concentration of H_2b_2 of approx 35 mg/ml to show this.

In the least squares approach, we need to scale the data to make the reference hemoglobin.

Our absorbance @ 577 nm is 2.451 ≅ 35 mg/ml
but the absorbance of the reference hemo is 1.436 ≅ 20.5 mg/dl
so you need to scale your spectrum by this ratio.

Concentration Hemoglobin ≅ 14.3

@ 577nm if $\frac{\log(y)}{\log(x)} = \frac{2.457}{1.436}$

If $\log y = 2.457$
$y = 10^{2.457} = 282.5$ $\quad \frac{y}{x} = 10.35$

$\log x = 1.436$
$x = 10^{1.436} = 27.29$

@ 446 @ 446 nm

$\log y = 2.266$	184.5	$\frac{y}{x}$	$= 6.15$
$\log x$ 1.477	29.99		

To the blood cultures:

To one of them I added NaOH, $FeSO_4 + H_2O_2$
(NaOH by mistake)
it seemed to form a filament base immediately
it also clarified the medium.
To the other I only added
$FeSO_4 + H_2O_2$; it did not have the
same effect.

OK, we know what we want to solve.
The concentration problem.
What is the concentration of the hemoglobin
and the concentration of the culture in affected blood?

We need to get hemoglobin reference chart in
the system.

340
340.5
341
341.5
342

344 – need this to be 342
344.5
345
345.5
346

1 340
2 342
3 344
4 346

Read 1, 2
Read 2, 3
Read 3, 4

i		
1	340, 41	Ok, I have hemoglobin data!
2	342, 42	
3	344, 43	
4	346, 44	

We now have a composite graph of
- Theoretical Hemoglobin @ some concentration
- The Culture @ some concentration
& Affected Hemoglobin @ some concentration.

Concentrations are:

λ

541 Affected: $2.451 = 35$ mg/ml

541 Pure Hemoglobin: $1.436 \cong 20.5$ mg/l (?)

446.5 Culture: $\frac{1.784}{.5491} = 3.25 \left(\frac{5\text{mg}}{l}\right) \cong \frac{16.2\text{mg}}{l}$

absorbance

Prahl

$A = \epsilon c d$ or

Thomas

$A = \epsilon C \ell$

$A = \log\left(\frac{I}{I_0}\right) = \log\left(\frac{1}{T}\right)$

$A = a\, b\, c$ (b: path length; c: grams/liter)

a: absorptivity (coefficient)

$A = \epsilon\, b\, C$ (b: path length; C: moles/liter)

ϵ: molar absorptivity (coefficient) → this is equivalent to the molar extinction coefficient.

A is A no matter where it comes from.

the unit for ϵ is $\frac{\text{liter}}{\text{mole} \cdot \text{cm}}$

it is a coefficient to make A come out to a ratio.

$A = \epsilon$ _____

$A_\lambda = \epsilon \cdot C \cdot \ln(10)$

Scale used is		log
	1000	3
	100	2
	10	1
	1	0

$\log(250) = .6$?

You now have some reference hemoglobin charts developing. This is looking very good.

You only have a problem w/ scaling. You have a log scale which looks real good (not sure how you got it.

First no on 150 mg/mL is 250.093
last no is

Seem like we should be plotting

~~$\log_{10}(A) = 1$~~
$\log 1 = 0$

$\log 2 = 1$

$\log 3 = 2$

$A = \log_{10}\left(\frac{1}{T}\right)$

$A = -\log_{10}\left(\frac{I_0}{I_a}\right)$

$\log(x-1)$

$-\log(.1) = 1$

$-\log .01 \quad 2$

$\frac{1}{T} = 2000$

$\log(x) = 1$

$\frac{1}{T} = 1 \quad \log(1) = 0$

$\log \frac{1}{T} = 10$

#T	1/T	−log (1/T)
1	1	0
2	.5	.3
3	.33	.48
4	.25	.60
5	.20	.70
10	.1	1.0
20	.05	1.3
50	.02	1.70
100	.01	2.00
200	.005	2.30
500	.002	2.70
1000	.001	3.00
2000	.0005	3.3

T	A (Log)	1/T
1	0	1
.1	1	10
.01	2	100
.001	3	1000
.0001	4	10,000

$A = \frac{1}{T^n}$

* Answer is $-\log\left(\frac{1}{y}\right) + 1$

where y is the magnitude of the actual absorbance as determined by $A = \varepsilon c l$

You have now made some significant progress.

You have good reference hemoglobin plots.

You have also learned of the relationship

$-\left(\log \frac{1}{y}\right) + 1$ is what you apply to the actual magnitude of absorbance. The log scale is a convenience from the instrument.

In addition you have a reasonable composite plot based upon actual data now.

We pick

$A_M = A_H + A_C$

H = reg. hemoglobin
C = culture

Hemoglobin Estimate is to high

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$C_x = .392$

$C_y = .71$

$C_x = 3.92$?

$C_y = 11.36$? 2.84

1 = Black
2 = Blue
3 = Red

What does this good solution actually mean?

340 $\frac{1}{35}(2.22)C_x + \frac{1}{16}(.680) = .799$ C_x

379.5 / 368.5 $\left(\frac{2.133}{10}\right)\left(\frac{1.18}{16}\right)4 = 1.245$ C_y 1

397 / 401.5 $\left(\frac{2.571}{10}\right)\left(\frac{1.793}{16}\right)4 = 1.069$

401.5 $\frac{2.637}{10}$ $\frac{1.183}{16}4$ 1.420

446.5 $\left(\frac{2.06}{10}\right)$ $\frac{1.734}{16}4$ 2.266

453 $\frac{1.94}{10}$ $\frac{1.379}{16}4$ 1.745

504 $\frac{1.5}{10}$ $\frac{1.324}{16}4$ 1.256

545 $\frac{1.898}{10}$ $1.069/16\,4$ 2.362

561.5 $1.704/10$ $.95/16\,4$ 1.843

577 $1.932/10$ $.853/16\,4$ 2.451

600 $.696/10$ $.724/16\,4$ .443

700 $-.347/10$ $.364/16\,4$ .219

This is stock Concentration by a factor of 3

We believe our hemoglobin concentration is too high by a factor of $\frac{35}{10} = 3.5$

We also believe that our culture estimate is also too high by a factor of $\frac{16}{4} = 4$.

Stock Solution = 1.25 mg/ml
Concentrated = 3.75 mg/ml

We believe our best model right now is

$$\frac{a_x}{C_{\text{net hemoglobin}}} \cdot C_x + \frac{a_y}{C_{\text{culture concentration}}} C_y = A_{\text{MINUS BG affected blood}}$$

* (1) So we think the concentrated blood solution is approx 10 mg/ml.

and that concentration equation should be

$$\text{Conc of Hemoglobin in } \frac{\text{mg}}{\text{ml}} \cong 4.09 * \text{Absorbance @ 577 nm}$$

(2) We think that concentration of "Stock" culture solution is approx 1.25 mg/ml

and that concentration equations

$$\text{Conc. of Stock Culture Solution in } \frac{\text{mg}}{\text{ml}} \cong \frac{\text{absorbance @ 446 nm}}{2.196}$$

Examples:
Assume hemoglobin affected measures at 2.5 Absorbance. Estimate of hemoglobin (2.5)(4.0) Concentration is: 10.2 mg/5ml
This is reasonable.

Assume culture stock solution measures at 0.7
Concentration estimate is $\frac{.67}{2.196} = \frac{.30\ mg}{ml}$
If it was 1.75
which seems closer to reality, it is $\frac{.80\ mg}{ml}$

Now if we assume blood has approx.
$\frac{150\ gms\ Hb}{liter} = \frac{.15\ gms}{ml} = \frac{150\ mg}{ml}$

We would need to multiply our results for real blood by a factor of:

$\frac{150\ mg/ml}{10.2\ mg/ml} = 14.70$

So hemoglobin estimate is ~ 150 mg/ml
Culture estimate is
from chart $\frac{2.0}{2.196}$ (~~14.70~~ 14.70) = $\frac{13.4\ mg}{ml}$ estimated in the blood.

5600 ml of blood in the body, so 5600 (13.4) ≅ 75 gms in the body.
≅ 150 of the petri dishes.
not bad.

We have a problem w/ culture estimate for a concentration.

We originally believed it was a concentrate of approx 5 mg/ml. This means our boiled down vessel has a concentration estimate of 15 mg/ml (5 x 3). But we believe we have overstated it by a factor of 4.

Therefore our latest estimate for concentrated culture solution is $\frac{15}{4} = 3.75$ mg/ml in the concentrate

and $\frac{3.75}{3}$ in the stock, or 1.25 mg/ml

Ok in the calibration of the stock concentrated stock solution we arrive @ relation

Absorbance = .5491 * Concentration ← this observed
→ that has to increase to be the relationship

this no. holds

but we thought the concentration was 15 mg/ml but now we think it is mg 3.75

So now our absorbance is

Absorbance = .5491(4) * Concentration
Absorbance = 2.2 * Concentration

So Concentration $\approx \frac{\text{absorbance @ 446.5 nm}}{2.2}$

We measure approx 2 on our graph
$\frac{2}{2.2} = \frac{\text{.9 mg}}{\text{ml}}$ estimated.

Examples:
Our concentrated culture solution measures approx 1.75 on our graph.
$\frac{1.75}{2.2} = \frac{\text{.8 mg}}{\text{ml}}$ estimated.

Our stock solution is estimated @ 1.25 mg/ml
so this is not unreasonable.

BUT:

Our least squares solution gives

$C_x = 3.92$ mg/ml
$C_y = 2.84$ mg/ml

Model is:

$A_{\text{affected}} \cong \underset{\text{Concentration}}{C_x} * 3.92 C_x + \underset{\text{Culture Concentration}}{C_y} * C_y$

I am confused on p130 between what is the reference concentration and the computed concentration. What is the difference?

We know that the concentrated solution come out very close to the concentrated blood. This is bad news.

The solution is saying that the contribution mass is fairly close between the hemoglobin and the culture. This is also bad.

We are definitely on the right track but we need to think about this a bit more.

---

matrix form

Solving $Ax = b$ has a straightforward approach

$$A_1 = a_{1,1} b C_1 + a_{12} b C_2$$

$$A_2 = a_{21} b C_1 + a_{22} b C_2$$

$$\vdots$$

this is the approach

$$\begin{bmatrix} a_{11} \cdot b & a_{12} \cdot b \\ a_{21} \cdot b & a_{22} \cdot b \\ \vdots & \vdots \end{bmatrix} \begin{bmatrix} C_1 \\ C_2 \end{bmatrix} = \begin{bmatrix} A_1 \\ A_2 \\ \vdots \end{bmatrix}$$

So the culture has concentration a
Blood has concentration b.

Now in theory you need to know a,
but you don't.
In theory you need to know b.
Now you mix them in any way you want
& they will need to make the
actual spectrum of blood. So you
do not know everything that is normal.
Our situation is:

C_x = standard
C_1 = mixture

$A_{11} = a_{11} b C_1 + a_{12} b C_2$

$A_2 = a_{12} b C_2 + a_{22} b C_2$

but $a_{11} = \frac{A_{11}}{b \cdot C_x}$ we do not exactly know this

so our equations are actually:

$A_1 = \frac{A_{11}}{b \cdot C_x} b \cdot C_1 + \frac{A_{12} b}{b \cdot C_y} C_2$ so b cancels

so primary form is
you have 4 unknowns, not 2!

$A_1 = \frac{A_{11}}{C_x} C_1 + \frac{A_{12}}{C_y} C_2$

How would you solve a system of equations like this?

$y = \frac{A_{11} \cdot X_1}{X_2} + \frac{A_{12} X_3}{X_4}$ great problem. This is what we need.

We could solve for the ratio to get it started.

We would know the

$\frac{\text{Culture in mixture}}{\text{Culture of standard}}$ $\frac{\text{Concentration of blood in mix}}{\text{Concentration in standard}}$

What if we formed this ratio?

$\frac{\text{Culture in Mixture}}{\text{Culture of Standard}} \cdot \frac{\text{Concentration of blood in Standard}}{\text{Conc. of blood in mixture}}$

$= \cancel{Cm \cdot S} \quad \frac{Cm \cdot Cs}{} \quad \frac{Cm \cdot Hs}{Cs \cdot Hm}$

You could solve them by trial & error since you do not know them by mixing them theoretically

So units for a·b·c matching the units for A (which is a ratio and therefore unitless.

If you don't have A (an actual ratio) then you have to work on solving the parts of it, which are a·b·c

Now what do we know and not know?

We measure the a, ·b somehow. How do we get this. from X/X, units for a are

liter / gm·cm also $a = \frac{A}{b \cdot C}$ ← this is what we measure!

this is freed for cuvette

So what exactly is this C compared to the C, we are solving for. Somehow it is the C of a standard solution of some kind. How & why? They have to come from a Calibration Curve.

* So you have to have controls over the two components of a mixture before you can analyze the mixture.

Ok now I understand the need.
Let's say you have A of Concentration C_a
Let's say you have B of Concentration C_b
Now when you mix them together in arbitrary amounts, you have no idea what you have. You might add 30% A, 70% B (but you don't even know this) you can hardly know what you have.

So what if we take a trial conc. of culture,
add it to theoretical blood
& try to create what results in actual blood.

So start out with any C.

We had a great solution method.
You will assume an arbitrary blood
spectrum.
You will take a trial culture
and get its spectrum.
You will find a solute that gets
as close to real blood as possible.

So in our trial,

Less culture, more blood?

ok, we can tell we need Stock Concentration 3
to produce the effect we need on
Concentrated Blood.

Black = #1 (Model) decrease
F Red #2 (Actual Blood) increase
H = Culture

What we are actually seeing is that the culture affects the spectrum more on the left side of the spectrum than the right.

1 = Black — 10 gms / Blood
2 = Red — Culture 3.0
3 = Green — Strong Blood Measured 10 gms estimated
4 Pink — Model of Strong Blood Measured.

Notice Red & Green are identical from 340 to 470-500
Then from 470 to 600 green & black are very close.

This means
340 to 470: Culture ⇄ Strong Blood Measured

470 to 600-500: 10 gms Blood ⇄ Strong Blood Measured.

This says the culture has a great deal of effect

So the strongest culture almost exactly matches measured blood in the 340 to 500 nm range.

This is the next effect.

So the effect of this is that the culture is "replacing the blood" in the portion of the spectrum 340-500 nm.

A Concentration of 3.0 is replacing a spectrum of blood @ 10 mg/1 ml in the range of 340 to 500 nm.
This is remarkable.

You will need to show the progression of concentration curves.

Culture notes:

- Initial growth in $FeSO_4 + H_2O_2$ to get started
- Followed by blood only progressed to a filament form
- Then added more $FeSO_4 + H_2O_2$ like 0 dose eliminated @ 4
- blue light
- The culture has really taken off and has also changed form back to E3 vs filament.

glass = .06 ml/drop
plastic = .065 ml/drop

Culture Extract Prep of Agar Farm from [redacted]

~~1.37 gms~~ 1.35 gms
100 ml of water to start
20 drops NaOH (glass dropper) (20 drops = 20(.06) = 1.2 ml

We will boil down to 50 ml
This is equivalent to $\frac{1.35 \text{ gms}}{50 \text{ ml}} = \frac{.027 \text{ g}}{1 \text{ ml}} \approx \frac{27 \text{ mg}}{\text{ml}}$

Now when we filter this
we are getting only a fraction of the actual mass.
We do not know what this is but we could estimate 1/10 of the mass.

An estimate of the concentration of the solution is therefore 2.7 mg $\approx \frac{3 \text{ mg}}{\text{ml}}$ Our current estimate

Your previous estimate was original $\frac{*3}{4} = \frac{5 \times 3}{4} = 3.75$

We can weigh the paper filter
after it is dry & subtract from a clean
filter to get the actual number!

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We now have some requirements

1. Start w/ 100 ml and 1.35 gms
2. Boil down to 50 ml
3. Drain & filter (Remaining liquid = 49 ml
4. This increases concentration to

$$\frac{1.35\text{ gm}}{49\text{ ml}} = \frac{.0275\text{ gm}}{\text{ml}}$$

but after we dry the filter and subtract a dry filter we will get remaining concentration.

$$= \frac{1.35\text{gm} - \text{filter residue}}{49\text{ ml}} = \frac{X}{1}$$

and this will be actual concentration.!

Filters weigh $\frac{5.06\text{ gms}}{3} = 1.687\text{ gms} = 1.69\text{ gms}$

Our dried filter weighs 2.07 gms

So the mass into filter is $2.07 - 1.69 = .38\text{ gms}$

glass ≈ .06 ml/drop
plastic ≈ .065 ml drop

Concentration of Stock Solution

Pase 18)

Now, for the first time, we can properly determine the concentration of a stock solution. Let's see how good our estimate was.

We have .38 gms = $\frac{.38 \text{ gms}}{49 \text{ ml}}$ = $\frac{.0076 \text{ gms}}{\text{ml}}$

100 ml − ~~50 ml~~ + 1.8 ml (NaOH)
−52.0 (boiling)

= $\frac{7.75 \text{ mg}}{\text{ml}}$

We estimated it to be between $\frac{3\text{-}4 \text{ mg}}{\text{ml}}$.

So this means $\frac{.30}{1.35}$ = 20% went into solution.

This is good work.

Set up Calibration Curve. Use 1.5 ml = 25 drops

	1.5 ml	Δ from 1.5
7.75 mg/ml	= 25 drops	0
5.17	17	8
3.45	11	14
2.30	7.	18
1.53	5	20

The solution solidified. (It has agar in it!)
This is not going to work well.
Pour it into a dish and save it.
It will mess up the cuvettes.

glass ≈ .06 ml/drop
plastic ≈ .065 ml drop

Concentration of Stock Solution

Page 181

Now, for the first time, we can properly determine the concentration of a stock solution. Let's see how good our estimate was.

We have .38 gms = $\frac{.38 \text{ gms}}{49 \text{ ml}}$ = $\frac{.0076 \text{ gms}}{\text{ml}}$

100 ml − ~~50 ml~~ + 1.8 ml (NaOH)
−52.0 (boiling)

= $\frac{7.75 \text{ mg}}{\text{ml}}$

We estimated it to be between $\frac{3\text{-}4 \text{ mg}}{\text{ml}}$.

So this means $\frac{.38}{1.35}$ = 28% went into solution.

This is good work.

Set up Calibration Curve. Use 1.5 ml = 25 drops

	1.5 ml	Δ from 1.5
7.75 mg/ml	= 25 drops	0
5.17	17	8
3.45	11	14
2.30	7.	18
1.53	5	20

The solution solidified. (It has agar in it!)
This is not going to work well.
Pour it into a dish and save it.
It will mess up the cuvettes.

Now we have 1.55 gms in 100 ml
Fine powder
Heated to boiling
Weget 97 ml back

Later on we need to weigh:

1. The 250 ml beaker dry w/ solids
2. Clean the 250 ml beaker
3. Weigh it & take difference
4. Weigh the dried fodder & subtract filter weight
5. Add up these
   in 97 ml water

6. Will give us the Concentration

For now use 1/4 = $\frac{1.55 \text{ gms}}{4}$ = $\frac{.3875 \text{ gms}}{97 \text{ ml}}$

= $\frac{.0040 \text{ gm}}{\text{ml}}$ = $\frac{3.99 \text{ mg}}{\text{ml}}$ ≅ $\frac{4.0 \text{ mgs}}{\text{ml}}$

Very close to original estimate.

=1.5ml

~~4~~ mg/ml	7.11	25	0	Max 2.5?	4/25 = x/17
-1/3 ~~2.72~~	4.74	17	6	Max 2.5?	
~~4.76~~	3.12	11	14	Max 2.5?	~~+3.01~~ 2.50
~~4.12~~	2.06	7	18		1.921
~~0.80~~	1.31	5	20		1.301

We do not have a good solution. Why?

We have a useful solution only w/ 5, 7, 11 drops.

Abs ≅ .2319 * Concentration in drops

or

$$\text{Concentration} = \frac{\text{Absorbance}}{.2319}$$

It is reaching a limiting value of 2.5 Absorbance - Why?

Concentration in mg/mL = Conc. in drops * ~~1.76~~ .284

Concentration in mg/mL = Absorbance (abs) = Absorbance * ~~.69~~ .8226
.2319 .284

This whole thing is very odd. Why does it reach a limiting value

Positively it is reaching a limiting value of 2.5. Why? Something is wrong here.

This is not a good solution. Wine Intolerance?

We have some kind of contamination that is raising the absorbance.

Even though it appears to be contaminated
lets see what the concentration is:

Weigh + filter = 2.01
Dried filter = 2.07
.14 in filter

We had a total of 1.55 gm to begin with:
1.55
− .14
.81 gms left

and leftover in flask ≈ .12 gms

so .81
− .12
.69 gms went into solution

$$\frac{.69 \text{ gms}}{97 \text{ ml}} = \frac{.00711}{1 \text{ ml}} = \frac{7.11 \text{ mg}}{\text{ml}}$$

This is still useful but it is contaminated in
some way.

Good for growth but not so for analysis.

We still might be able to use it
to calibrate the original solution
however

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We can compare the other to it
and get a good concentration no.
using max peak @ 446.5

Let's translate what we know about one
stock solution to the other. We have
one good solution we know to be at 7.11 mg/ml
for some reason it reads @ Abs = 2.5
Let's set a concentration which is less than 2.5
We have

Conc (mg/ml) = Abs * 1.226 @ 446.5 is max

Read 1.824

So Con = 1.824 (1.226) = 2.24 mg/ml

Now compare this to stock solution #1 ~~Conc .556 mg/ml~~

Absorbance @ 446.5 = .556

.556 (1.226) = .682 mg/ml

Next trial 108

2.93 gms of black tea 100 ml H2O ml
20 drops NaOH

We have 105 ml after drain.

Need to subtract residue in flask and filter remainder.

Ok, we have a good solution now

	Δ
391	50.6
441.5	50.0
491.5	49.5
541.0	

5 drops
25 water
] total 30 drops

looks very good.

All we need now is the weight.

10 drops
vs 15 water
] 30 looks decent

15 vs
15
] 30 Still works fine but is broadens the peak considerably.

We can make initial estimates, before we can weigh everything.

@ 446.5 we have relations

Conc (mg/ml) = Abs. (1.226) = 2.

	Abs	Conc
5 drops	1.591	1.95 mg/ml
10 drops	2.007	2.46 mg/ml
15 drops	1.946	2.39 mg/ml

Linearity of Beer's Law may be violated @ higher concentrations a high concentration may also indicate errors.

Then is our first estimate. It will be refined.

Flask contribution = 0.39 gms
Dried Original Filter = 2.07 gms
Notice we have peaks occurring @ 50 nm intervals!
Dried Filter = 4.78
We can estimate for now that ½ remains in filter
= 2.93 (½) ← 1.46 filter strike
+ .39 flask
1.85

2.93
− 1.85
1.075 gms

estimated.

$\frac{1.075 gm}{108 ml} = \frac{.00995 gms}{ml} = \frac{9.96 gms}{ml} \approx \frac{10 mg}{ml}$

A very reasonable number.

So now our estimate is 10 mg/ml

Our estimate based on 10 mg/ml

30 drops
1.8 ml

5 drops = 5(.06) = .30 ml · 10 mg/ml = 3 mg/1.8 ml
10 " 10(.06) = .60 ml = 6 mg/1.8 ml
15 " 15(.06) = .90 ml 9 mg/1.8 ml

Total mean
30 drops 5 drops / 1.8 ml = 1.67 mg/ml
30 drops 10 drops / 1.8 ml = 3.33 mg/ml
30 drops 15 drops / and 1.8 ml = 5 mg/ml

The looks very reasonable.

Later wgt of filter

4.76 gms wgt of filter dried
− 2.07 wgt of original filter
= 2.69 Material on filter
+ .39 flask
~~2.30~~ gms 3.08 flask + filter

and 2.93
− 2.30
= .63 gms

NO! Is more than original. Filter cannot be dry.

Blood:
29ml 576nm
@ 414 nm my reading ≈ ~~1.6~~ 1.92
Conc of Hemoglobin = ~~1.43~~ (1.92) = ~~21.5~~ mg/ml
4.09 7.85

5600ml of blood (about 3mg/ml) = 16.8gms

@ 2.93 gms for 2 petri dishes ≈ 11.5 petri dishes in body.

Ratio of Culture to Hemoglobin ≈ 3mg/ml / 7.85mg/ml = 38% by mass

---

Latest filter measure 3.29gms Now 3.07 05/31/11
- 2.07
1.22gms material on filter

So 2.93 original wgt + .39 beaker
- 1.61 ext material 1.61 material wgt extend to liquid
= 1.32gms
in Solution

1.32gms / 108ml = .0122 gms/ml = 12.22 mg/ml actual good msmt.

Adjustment 5/31/11
3.07 - 2.07 = 1.00 + .39 = 1.39
2.93 - 1.39 = 1.54

1.54 / 105ml = .0147 gms/ml = 14.67 gms/ml

gels, leave it as 07/08/11.

Proper Concentration Development of Stock Solution

Original mass of preliberged culture 2.93 gms
+ 30 drops NaOH (can be neglected)
+ Heat.

↑ not for conductivity! 07/08/11

We have 105 ml of tea filtered

Mass of dried filter 3.29 gms
Mass of dried filter 2.07 gms
mass of filter 1.22 gms
Remaining in beaker .39
Extract mass 1.61 → somewhere found it yes on previous later this page. must have since to 1.38??

Original mass = 2.93 gms
− 1.61
1.32 gm

07/08/11

In 105 ml
$= \frac{1.32 \text{ gms}}{105 \text{ ml}} = .01257 \frac{\text{gms}}{\text{ml}} = 12.6 \frac{\text{mg}}{\text{ml}} \approx \frac{12.5 \text{ mg}}{\text{ml}}$

Now 14.67

This is Concentration of primary stock solution.

30 drops =	Now Drops	Δ to 30	Mass of Drops
1.8 ml	5	25	5 (.06) = .30 ml (12.5 mg/ml) = 3.75 mg/ml
1.8 ml	10	20	10 (.06) = .6 (12.5 mg) ml = 7.5
1.8 ml	15	15	.9 (12.5) = 11.25

This should be solid.

Calibration Graph for Stock Culture

Now let's graph abs. = f(concentration)
w/ Calibration graph.

	Drops	Δ to 3	Conc mg/ml	(= 0.49 mg/per drop)
30	2	Δ28	.83 mg/ml	.98
30	5	25	2.08 mg/ml	2.44
30	8	22	3.33 mg/ml	3.91
30	12	18	5.00 mg/ml	5.86 Limit of work.
	15	15	Limiting Absorbance reached.	7.33 mg/ml
	20	10		9.78 mg/ml

We reach a limiting absorbance, which is what we found before.

We have a very good fit for 1st 4 data points.

Absorbance = .1962 * Concentration + .2943

or

$$\text{Concentration (in drops)} = \frac{\text{Absorbance} - .2943}{.1962}$$

$$\text{Concentration in mg/ml} = \frac{\#\text{Drops}}{30}(12.5) = \#\text{Drops}(.42) \quad (14.67 \to .49)$$

$$\text{Concentration in mg/ml} = \left(\frac{\text{Absorbance} - .2943}{.1962}\right)0.42 \quad (50 \to .49)$$

= 2.08 mg/ml
4.17 mg/ml
6.25 mg/ml

$$\text{Concentration in mg/ml} = \left(\text{Absorbance} - .2943\right) * 2.14 \quad (2.49)$$

@ 446.5 nm

Usable equation now.
We now have 4 Concentration Curves.

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Now lets look @
the Concentration Curves
We now have a theoretical model
for Leroy Labs.
Process:
1. Make an estimate of the Concentration
of Culture by using a measured blood
Vat 446.5 nm
and using relation
Conc (mg/ml) = (Absorbance − .2943) * 2.49 ~~2.14~~

2. Make an estimate of Concentration on Hemoglobin
C 576 nm by using theoretical blood
Curve.

3. Now find a model that satisfies
$A_M = A_1 + A_2$

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Example:
Culture Concentration Estimate
~~with Excised Blood~~, Abs @ 447 = 1.989
So estimate Culture Concentration = 5 2.49 4.22
Est Concentration culture = $(1.989 - .2943)(2.49) = 3.~~63~~$ ~~mg~~/ml

Blood @ 576: Abs = 1.918
Theoretical Concentration = 1.935 very close to 10 mg/ml

Theoretical Model $A = -\log_{10}\left(\frac{1}{(\text{Conc.}/64500 * \text{Molar Absorbtivity})}\right) + 1$??? n close to Beer's

So we have Concentration
Estimates of both solutes.
Now set up mixed model:
Meas
Blood = Hemoglobin + Culture

λ
447 $1.989 = \frac{2.017}{1.935}C_x + \frac{1.984}{3.63\ 4.22}C_g$

576 $1.918 = \frac{1.918}{1.935}C_x + \frac{1.935}{3.63\ 4.22}\frac{1.310}{}C_g$

$C_x = 10.016$ mg/ml
$C_g = -.05$ mg/ml
} not a very good solution.

We must go to least squares next.

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Est. 10mg/ml → Est. 3.63 → 4.22 → you need to change this to ~~5.00~~

	Hemoglobin	Culture	f
340	2.223	.73	.19
397	2.571	1.874	1.664
401.5	2.637	1.331	1.341
447.5	2.032	2.033	1.989
509.5	1.492	1.69	.981
542	1.917	1.502	1.943
559.5	1.707	1.344	1.405
576	1.935	1.272	1.918
598	.844	1.161	.434
700	0	.44	.244

$C_x = .324$ → 3.241 mg/ml

$C_g = .561$ → 2.036 mg/ml

$$S_{uA_{Blood\ meas}} \approx .324\left(\frac{\text{Hemoglobin A}}{10\text{mg/ml}}\right) + .561\left(\frac{\text{Culture A}}{3.63}\right)$$

$$\approx 3.241\left(\frac{\text{Hemoglobin A}}{10\text{mg}}\right) + 2.036\left(\frac{\text{Culture A}}{4.22}\right)$$

$$\approx 2.367$$

$\sigma \approx .5$?

Not too bad, you are on the right track!

If we change the assumed concentration of Leb culture to 5.0 from 3.63 then
$C_x = 3.241$
$C_y = 2.804$

This does give a slightly better solution.
$\frac{2.804}{3.241 + 2.804} 2.37 = 46\%$ ~~46~~ 42%

We create a model that most closely matches the measured spectrum and then tells you what the expected concentration is.

Unfortunately these results are not favorable Absorption flattening is also important. by design?

Your results on a mixture vs the original concentrations of the components is very similar to Thorpe pBQ. But it still is not clear to me why a mixture is different or especially less in total mass.

How can this be?

$-\log_{10}(5) =$ Theoretical Concentration of Blood (Hemoglobin)

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We do see that the concentration of the blood makes a very big difference in the spectrum according to the patterns that have been identified.

Sample today on blood 576 nm = 2.29 Abs.

We know that:

$$\text{Absorbance} = -\log_{10}\left(\frac{1}{\left(\frac{\text{gms/L}}{64500}\right) * 55540}\right) + A$$

So

$$-\log_{10}\left(\frac{1}{\left(\frac{\text{gms/L}}{64500}\right) * 55540}\right) = A - 1$$

$$= e_{10}^{(1-A)}$$

$$\frac{1}{\left(\frac{\text{gms/L}}{64500}\right) * 55540}$$

$$\frac{1}{\frac{\text{gms/L} \cdot 55540}{64500}} = \frac{64500}{(\text{gms/L})\,55540} = e_{10}^{(1-A)}$$

$$\text{gms/L} = \frac{64500}{e_{10}^{(1-A)} \cdot 55540}$$

$$\text{gms/L} = \frac{1.161}{e_{10}^{(1-A)}}$$

10

Concentration of blood given Absorbance @ 576

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eg 576: A = 2.29

Theoretical $Sm/L = \frac{1.161}{1-2.29} = 22.64$ mg/mL This is very reasonable

Reference $10^{\quad}$

$gms/L = \frac{1.161}{10^{(1-\frac{2.29}{A})}}$

$gms/L = \frac{1.16}{10^{(1-A)}}$

yes

Measured So we estimate our current Concentration as 22.64 mg/ml

Now we collect the data.

For cuvette @ 446 nm A = 2.083

Make up a proper cuvette

446 Concentration = (Abs − .2943) · 2.49 = 4.454 mg/mL

	Hemog (22.6A)	Culture (4.45A)		(Used 4.454/1.99 mg/drop = 9.1 drops Measured Blood
340	2.761	2.578	.697	.767
391	3.115	2.926	1.67	1.693
401.5	3.181	3.114 2.992	1.234	1.304
441.5	2.590	2.386	1.962	2.067
509.5	2.036	1.841	1.759	1.826
492	2.098	1.909	1.846	1.869
544.5	2.451	2.262	1.554	2.327
576	2.479	2.29	1.290	2.29
598	1.300	1.199	1.163	.79
700	.191	.008	.268	.299
453	2.484	.000 2.295	1.467	1.445

Solution is Cx = 2.523 Cy = 4.236 = 63%

Two modified sets of measured blood have been completed.

The result is rather profound.

* The greater the concentration of hemoglobin, the greater the impact of the organism upon the spectrum of the blood.

* We can also say the greater the relative mass of the organism to the total mass (hemoglobin + organism) the greater the impact upon the spectrum of hemoglobin to where it eventually dominates the spectrum.

Each solution takes about 2 hrs.

Now here's a question: How can you have varying levels of relative concentrations with a varying concentration of blood?

eg 42% & 63%? why?
Maybe we have error in the process and the average is our best estimate.

So what is the error in our process?

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Standard error of the unknowns

Model is

λ_i $\quad \Delta = \frac{a_x}{C_x^*} \cdot b \cdot C_x + \frac{a_y}{C_y^*} \cdot b \cdot C_y$

We need the errors of C_x & C_y then would be the standard errors of the unknowns

$Q_{\Delta\Delta} = N^{-1} = (B^t f)^{-1}$

and $\Sigma_{\Delta\Delta} = \sigma_0^2 \cdot (B^t f)^{-1}$ $\qquad \hat{\sigma}^2 = \frac{\Sigma v^2}{n}$

This is great.
and σ_i = the square root of each term on the diagonal.

Example: For our most recent solution of

$$\Delta = \begin{bmatrix} 2.253 \\ 4.236 \end{bmatrix}$$

we get $Q_{xx} = \begin{bmatrix} .604 \\ .175 \end{bmatrix}$ Now we need to multiply this by $\sigma_0 = \frac{\Sigma v^2}{n} =$

$\frac{\Sigma v^2}{n} = \frac{1.34}{11} = .122$

So $\Sigma_{xx} = .122 \begin{bmatrix} .604 \\ .175 \end{bmatrix} = \begin{matrix} .0736 \\ .0213 \end{matrix}$ $\quad \sqrt{.0736} = .27$ $\quad \sqrt{.0213} = .15$

Eqo
.44
.25

So this would lead to a potential error of $\frac{2.253 - .44}{2.253 - .44 + 4.236 + .25} = 29\%$

a Try: $\frac{2.253 - .27}{(2.253 - .27) + (4.236 + .15)} = 31\%$

so our function is $\frac{C_x}{C_x + C_y}$

What is error in this function?

$y = C_x \cdot (C_x + C_y)^{-1}$

$y' = C_x$

Still working on it.

$$\frac{4.236 - .25}{(4.236 - .25) + (2.253 + .44)}$$

$= .60 = 60\%$

So your first solution was 42%
your second solution was .63%

The expected error is on the order of 5%
The guess is an average of approx 52%
w/ an expected error of +/- 5%

The is quite high.

Now what are other people?

These are very large numbers

$\frac{BAD}{GOOD+BAD} \cong 52\%$ by relative mass.

What do these kinds of number mean in the blood?

So in our mixture, we have
$\frac{4.22 \text{ mg/ml culture}}{10 \text{ mg/ml hemoglobin}}$ or $\frac{4.45 \text{ mg/ml culture}}{22.64 \text{ mg/ml}}$

These number are based upon reading reading @ 446.5 and 576 specifically.
This is not totally unrealistic

The indicates it reaches a limiting concentration.
$\frac{4.22}{10} = 42\%$ $\frac{4.45}{22.64} = 19.65\% \cong 20\%$

5600 ml in blood.
150 mg average
$150/10 = 15$
Culture $15(4.22) \cong 63.3$ mg/ml
hemo 150 mg/ml

$150/22.64 = 6.62$
$6.62(4.45) = 29.5$ mg/ml
150 mg/ml
$= 165$ gms culture in blood

Est 354 gms Culture in blood

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Estimate of Concentrations:

[redacted]

2.4	$1.161 / 10^{(1-2.4)} =$	29.2 mg/mL
1.9	$1.161 / 10^{(1-1.9)} =$	9.2
2.0	$1.161 / 10^{(1-2.0)} =$	11.6
1.9	$1.161 / 10^{1-1.9} =$	9.2

[redacted]

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Let's look how food dye & the cell/tissue interact.

You can successfully simulate the situation w/ food dye & Culture.
1 drop red dye in cuvette
4 drops culture in cuvette.

Original red dye peak is ~ 1.41 @ ~ 520nm
4 drops of culture ~~bacteria~~ introduces the problem.

391
449 52
498 57

Many similarities

Interesting Work: Page 204

Yellow Food Dye is Complicated.

1. It also has an extremely sharp peak at 448nm.
2. The scratches in the plastic may be causing the artificial peak @ 397.
3. Scattered cuvette w/ yellow dye produces a spectrum amazingly close to the culture spectrum. This really complicates the picture you wanted to use to create a spectrum similar to hemoglobin, but no such luck.

Put [redacted] blood in a new container.

OK Results are duplicated. Primary Attributes are Clipper @ 2.5 on the left side of the spectrum & almost total absorption by the secondary peaks which gives blood its red color.

What is also very strange. The sample was split in half. The second portion settled & precipitated solids @ the bottom of the test tube. Run the solution through the spector and almost the entire spectrum was clipped @ 2.5. This indicates the blood has been transformed & completely demonstrates by high concentration of the organism.

You have run into difficulty that you are not able to stimulate hemoglobin very well as yellow would be to the chemical peak @ 440 so it is similar to the culture. Somehow you need to get a peak near 414.

Purple	Blue	Green	Yellow	Orange	Red
400	450	525	575	625	700

Hemoglobin has peaks @ 414 & 576
Purple Yellow Orange

Purple is made with red & ~~pure~~ blue
yellow is made of yellow

You do not appear to be able to simulate hemoglobin at the time. You would need a peak @ 414nm - how & where.

If something absorbs @ 400 it will be a dark red solution. So Color of solution is in opposition to absorption.

Hg Important discovery: We may have a "Raman water" peak of ~~341~~ 397nm that is distorting results.

Signs of greater effect from the bg correction are

1. higher relative peaks @ 397 nm or 446
2. Clipping @ 2.5
3. Lack of sharp drop off in 450-500 nm
4. Lack of strong peaks ~540 to 576 nm
5. Lack of sharp drop off after 576 nm
6. shifting of primary peak @ 446 ~448 nm

We developed a strategy of analyzing multiple spectra

1. Normalize all spectra.
   1. Determine Concentration of each spectra @ 576 nm
   2. Scale everyone to the average?

$$mg/ml = \frac{1.161}{10^{(1-A)}}$$

Deviation from Mean

Page 207

$\lambda = 596$

Scaled $\lambda = 446$

$1.161 / 10^{(1-A)}$

-12%	[redacted]	2.438	31.8 mg/ml	$2.496(.796) = 1.99$
-2%		1.921	9.7	$(1.94/1.921)(2.19) = 2.21$
-2%		1.911	9.5	$(1.94/1.911)(2.176) = 2.21$
-4%		2.021	12.4	$(1.94/2.021)2.254 = 2.16$
+20%		1.779	7.0	$(1.94/1.779)(2.496) = 2.72$

$\bar{x} = 14.1$ $\qquad$ $\bar{x} = 2.26$

$\sigma_{n-1} = 10.1$ $\qquad$ $\sigma_{n-1} = .27$

Now how could you scale the data for each plot.

Ex 10.1:

$$\frac{14.1}{10.1} = \frac{1.161}{10^{(1-A)}} \qquad 10^{(1-A)} = \frac{1.161}{10.1}\,14.1$$

$$1 - A = \log_{10}\left(\frac{1.161}{10.1}\right) \Rightarrow +A = 1 - \log_{10}\left(\frac{\frac{1.161}{10.1}}{14.1}\right)$$

$$= 1.94 \quad 2.1 \qquad 14.1 \qquad 2.1$$

$$\frac{1.94}{2.438} = .796$$

So in mind you would scale all values by Scaly factor

Use looks like a simple effective method of determining who has the most deviation from the mean.

The method developed is:

1. Estimate the hemoglobin content of the sample @ 576 nm by the relationship

$$\text{Concentration in mg/ml} = \frac{1.161}{10^{(1-A)}}$$

2. Find the average concentration of the group.

3. Solve for A corresponding to this average concentration.

4. Scale the max culture peak absorbance by the normalizing ratio:

$$\text{Mean } \frac{\text{Absorbance @ } 576 \text{ nm}}{\text{Measured Absorbance @ } 576 \text{ nm}} \times \text{Meas. Abs @ } 446$$

5. Determine the mean of this A Normalized & Scaled Absorbance value for each individual.

6. Determine the mean deviation in % terms for established relative influence of Culture in the blood of the sample.

Results appear to be quite realistic.

A Model to estimate the influence of the Magellanic Condition in a relative sense. "A Risk Model"

Page 209

Next stage is to determine the mean deviation from the entire spectrum. But you must normalize each spectrum first.

Ok you have done fantastic work.

You now have a method of evaluating deviations from an "average spectrum".

- High Risk
- Expected Range
- Low Risk

It clearly reveals the problem.

Results: Δ $100-(-\Delta)$ $-$SIGNA $\cdot$ $100+\Delta$

Age		Δ	Rank	$\frac{1}{2}-\Delta$ Age	Rank	Ranked
58	[redacted]	-31%	1	7.6	5	1
56?		-4%	4	1.9	4	4
62?		-16%	3	2.6	2	3
29		-17%	2	2.7	3	2
60		+68%	5	1.5	1	5

A qualitative health model estimate

$\frac{+\Delta}{(Age)^{1/2}}$ = Overall Score

I am making qualitative / quantitative health just assessments - you must be careful here even if it is true —

Scaled to 100.

Ranking: [redacted]

+47
+36
+23
+6
−100

Probably need to keep to self.

What can be discussed w/ the public?

Page 210

There is another way of thinking about this:

Deviations from the average are a problem?
(But what if everyone is in terrible health)?

Or High Deviation are problematic?

Low Deviations are beneficial?

~~We Can~~

We have the mean absorbance of Concentration of the group of six individuals as 15.3 mg/ml. By our formula

$$\text{Absorbance} = 1 - \log_{10}\left(1.161 / \text{Concentration in mg/ml}\right) = 2.1$$

leads to absorbance = 15.3

By our spreadsheet we get 14.5 mg. So 15 mg/ml is very close. Which is = 2.1 This is good. We are ok.

Now we have a reference spectrum of 516 nm ⇒ A = 2.1 ⇒ 15 mg/ml

All spectrums of the individuals are scaled to the concentration level of 15.3 mg/ml.

Now you can see the problem very clearly The reference hemoglobin chart @ 15.3 mg/ml vs the 6 individuals scaled to the same concentration.

Now lets look at deviations from the reference by individual.

Rank of Concern, based upon deviation from reference H is:

	ΔRed		ΔAbs
[redacted]	100%	[redacted]	100%
	66%		54%
	87%		38%
	71%		33%
	66%		31%
	66%		15%

This appears to be the best analysis thus far?

The question is, What do you want to adopt as your reference? Normal hemoglobin in all health?

Logic would say Reference Hemoglobin - -

The numbers here are bad.
Blood has been altered in a significant fashion.
Extremely low for the left 1/3
Extremely high in the right 1/3

The high risk candidate seems to be the person that is higher than the average deviation from the reference.

You do not have enough data yet to know who is a high risk star riders.

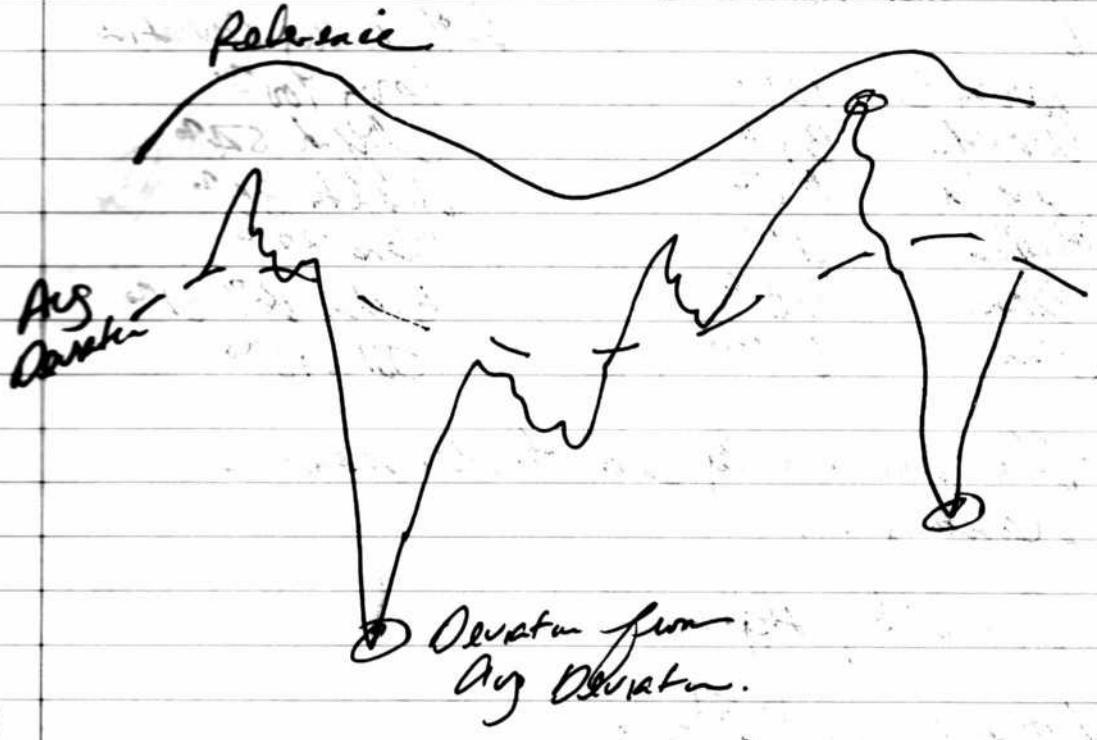
It looks like we now have a method of isolating the outliers

We take the avg deviation from reference hemoglobin.

Now you look at the individual differences from that avg.

Outliers are candidates.

We can only identify outliers at this stage.



$$X_i - (\bar{D} - R)$$

Now if someone's deviation greatly from the avg deviation, could they not be moving toward the reference hemisphere.

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In absolute terms of ranking deviation from hemoglobin

[redacted]
100%
89%
87%
71%
60%
67%

Ref is by deviation

[redacted]
100%
54%
30%
33%
31%
15%

These are actually close to the same rankings.

Scaled by Age:

[redacted]
100%
64%
56%
38%
32%
17%

This looks very reasonable

Quite a bit better

to be

This seems the best result.

Risk Equation $= \frac{\left(\frac{\Sigma V^2}{n}\right)^{1/2}}{AGE^{3/4}}$

$V = \left(X_i - \overline{X_e}\right)^2$

X_i = measured absorbance at λ_i

$\overline{X_e}$ = Avg Deviation from Reference @ λ_i

Lesson!

There is a very big difference in the spectrum depends upon concentration. If you get the solution too concentrated you get a very different spectrum.

You definitely do not want the 5% to read to be much above 2. It creates a problem.

I am getting a very big difference in the results w/ the new Ocean.

[redacted]	100 to 96	7.0		1.779
	69 to 65	12.4	Does not seem to correlate w/ concentration very well. There is good.	2.027
	39 to 38	9.5		1.911
	38 to 35	22.8		2.294
	26 to 25	7.0		1.783
	22 to 21	9.1		1.921

a bit better
* abit worse
* got the same
good?

Highest risk candidates are determined. It also does not seem to correlate w/ absorbance. The is also good.

Current Ranks: 06/01/11

[redacted]	96 96
	65 64
	38
	35
	25
	21

Mean absorbance @ 511nm = 2.0
Maybe a bit high for ocean

Results 06/08 Page 216

[redacted] 96
62 high score primarily due to the age.
37
36 ~~This prediction not so good.~~ Very interesting not so bad after all!
35
2
0

ok thus far

What to do about this?

10 electrons

H (1)	(1) bond	
N (5)	(3) bonds	H – C ≡ N:
C (4)	(4) bonds	
= 9		

In the "normal" individual the blood has been changed but not at the point that transfers skin symptoms.

Those that manifest @ the skin level are expected to deviate from the norm.

Two categories:
1. Those that are more likely to exhibit specific symptoms
2. Those that are may be highly susceptible candidates for displaying symptoms (Considers age as a factor)

Australia Report

$\frac{.05B\ gm}{m3} = \frac{5B\ mg}{m3} = \frac{5mg}{(100cm)^3} = \frac{5mg}{100\ 1E6\ cm3}$

$= \frac{5mg}{1E6ml} = \frac{5E-6\ mg}{ml}$

$.2ppm = \frac{.2gms}{1E6gms} = \frac{.2gms}{1E6\ cm^3} = \frac{.2gms}{1E6ml} = \frac{X}{1ml}$

$X = 20\%\ \frac{2E-7gms}{ml} = \frac{2E-4\ mg}{ml}$ "Safe limit"

and $\frac{2E-4\ mg/ml}{5E-6\ mg/ml} = 40$ times legal limit is 40 times greater than that measured

Queens Land Report

$320\ us/L = \frac{320E-6gms}{1000ml} = \frac{X}{1ml}\ .0003.2mg$

"safe" limit is $\frac{2E-4\ mg}{ml}$

$= \frac{3.2E-4\ mg}{ml}$ = 1.6 "safe limit"

so indeed this is slightly higher.

CALIF allows $\frac{1000ug}{Liter}$

Age-Risk Magnitude Rank	Magnitude Age-Risk Rank
[redacted]	[redacted]
96	100
62	41
37	38
36	37
35	23
22	18
18	18

We may have something here.
Fe^{+2} looks like it has a peak @ 397 also?

NaOH + Fe^{+2} gives dark green
Fe^{+3} gives dark brown

If you add peroxide to Fe^{+2} it will convert it to the brown form Fe^{+3}

$Fe^{3+} + 3OH^-_{(aq)} = Fe(OH)_3$ iron(III) hydroxide

dissolves readily in HCl & H_2SO_4

Fe_2SO_4 + NaOH → green ($Fe(OH)_2$)

$Fe(OH)_2$ + H_2O_2 → $Fe(OH)_3$

$Fe(OH)_3$ dissolves in HCl

You have solved an essential problem.

You have learned that a primary, if not the primary component of the organism is iron oxide.

You have proven this by developing the spectrum for iron (iii) oxide through a chemical reaction. Comparing it to the culture you see that it is essentially identical.

Chemical reaction to produce iron (III) oxide

$FeSO_4 + NaOH \Rightarrow Fe(OH)_2$

$Fe(OH)_2 + H_2O_2 \rightarrow FeO(OH)_3$

This is all astounding. You have proven the role of iron. This also can give you a concentration level of the culture for the first time.

So now you have a reference chemical and a reference culture concentration.

Let's Construct $Fe(OH)_3$ w/ the tablet form via the prepared solution.

Results have also been proven with a tablet form of $FeSO_4$
Concentration is weak but we pick up 1st 2 peaks @ 397 & 447 nm.

Results are proven

709 eV from a source corresponds to $Fe_2(OH)_3$
530 eV

$\lambda = hc/E = \frac{1240\ eV \cdot nm}{709\ eV} = 1.74\ nm?$

$\frac{1240\ eV \cdot nm}{530} = 2.34\ nm?$

We now have a means of estimating the concentration of the dalteen.

20 ml H_2O

20 drops .5 M $FeSO_4$

42 drops NaOH 1.0 M

4 drops H_2O_2

gives peak @ 391 of 1.210

You should be able to get concentration now.

We have

391

441

~~490~~ This is not definitive

You now have confirmed iron oxide(III) from a pure source. The test work is valid.

What books do you want to bring?

The next thing we learn is that iron is only 0.33% of the mass of hemoglobin.

There are 4 ions of iron (Fe^{+2}) in one molecule of Heme.

100 gms Hemoglobin → translates to → .33 gms Fe

translate to molecule

moles of Fe = $\frac{.33 \text{ gms}}{55.85 \text{ gms/mole}}$ = .00591 moles of Fe

in 100 gms of Hemoglobin.

So the first thing going on is that we are assuming all the iron in hemoglobin is ionized in form of Fe^{+2}

1. Yes a heme group contains an iron ion. It must be in the +2 state to bind Oxygen

Now in our solution, how much is ionized?

Ours is solid

Does our solution of culture settle?

Do not overwrote a .CSV file. It did not do it!

Question: Does iron oxide exhibit clipping? 397 448

Cannot really tell. I can not get the concentration high enough to show it. At this point the answer is no.

You can study the reaction of $FeSO_4$ w/ Stoichometry –

Meas	Theretical	Avg Intensity	Energy Level
397	397.1 = 391	80	I
401	401.2 = 401	1200	I
443.5	442.8 = 443	600	I
448	448.2 = 448	200	I

These are spectral lines of neutral (I) and singly ionized (II) atoms.
doubly ionized (III)
triply ionized IV

$Fe.SO_4$

$Fe^{+2} + 2OH^- \rightleftharpoons Fe(OH)_{2(s)}$

$Fe^{+3} + 3OH^- \rightleftharpoons Fe(OH)_{3(s)}$ Iron III hydroxide

here it is ionized — it is neutral

$\lambda = \frac{1240}{eV}$

Could we get back to intensity concentrations?

You know that

have potential of cookie.

iron oxide III + reference hemoglobin $\tilde{\Rightarrow}$ measured blood.

have an estimate of cure.

also
Culture + ref hemo $\tilde{\Rightarrow}$ measured blood.

$$\begin{array}{ccccc} X & + & y & = & z_a \\ U & + & y & = & z_b \end{array}$$

$$\frac{X+y}{U+y} \tilde{=} 1$$

so $X+y \tilde{=} U+y \quad \Rightarrow X \tilde{=} U$

Lets see if $\frac{I}{I}$ had asorbance
log problem worked at right

We had: $-\log\left(\frac{1}{y}\right)+1$ ← why did you add one? So what is y?

We also had

$A = -\log\left(\frac{I}{I}\right)$

1/T	T	A
1	1	0
10	~~01~~ .1	1
100	~~001~~ .01	2
1000	~~0001~~ .001	3

The problem came from the optical absorbance of hemoglobin paper when he gave absorbance in a value of essentially 0 to 2000 instead of by units. The only way I could get this scale to work was with a transformation of the order.

$$A^* = -\log\left(\frac{I}{I_0}\right) + 1$$

Now lets look at it more closely.

2000
1000
500
100
10
5
2
1
.5
.1
.02 → -.70
~0.

So this is my transformation which may not be exact but it truly does seem very close

So you are close, but your 0.1 is transforming to 0, not 0 transforms to zero.

I have fixed the problem, even if improperly by translating the data ≥ 0. I understand that the solution is imperfect but it is sufficient for now. We acknowledge that we have some scaling error.

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Hydrogen has a 397 absorption

Iron oxide has an absorption @ 397.0

Harris p410 shows us formaldehyde has an absorption of 397 nm.

He has one @ 396.5

Li has 396.5

Nothing in Beryllium

Sodium No

Mg 396.7 so it is breaking down.

Characteristics of the Organism (spectrum) *

We know now at least some things about the likely constitution of the organism:

1. heteroatom CN^-, CO Sept Oct 2, 2011
2. $n \rightarrow \pi^*$ bond (CO, CN^- Oct 2, 2011)
3. free electron pair is linked to another atom by a multiple bond
   eg $C\equiv N$ $C=O$ $C=S$ Can Iron or Hydrogen add to these?
   Oct 2, 2011
4. Within the "R" band
5. Matches the spectrum of Iron (Hydroxide) $Fe(OH)_3$
6. A Organ candidates in the Japanese paper. (what about inorganic?)
7. Formaldehyde is CH_2O has peaks @ 205 & 397. Hydrogen also has peak @ 397.
8. Energy required is $E = hc/\lambda$ $h = 6.626E-34$ λ in meters

   $E = \frac{hc}{\lambda} = \frac{1240}{\lambda_{nm}}$ $\lambda = \frac{hc}{E} = \frac{1240}{3.2}$ $C = 3E8$ m/sec

   $E = 3.12$ eV $= 381.5$ nm
9. These transitions require need an "unsaturated group" in the molecule to provide the π electrons. bond bunk class. Com

Page 228 Solution needs to make up w/ iron hydroxide

Page We have 12 candidates showing up

40 $N-(N=C)-C=C-C$

No 131 $6-C\equiv C-C\equiv C-C\equiv C-C\equiv C-6$

No 131 $6-C\equiv C_5-6$

? 182 $O_2-6-C=C_{O_3-6}>C=O$

317 $^{6}6$

339 $C-N6=O$

456 $C_2-6-O=O5=C-6$

456 $C_2-6-O=O5=C-6$ Same

456 $O-6-O=O5=C-6$

461 $O=O_2^{|}O65=C-6-O_2$

479 $O_3-6-C=ON5=O-6-N=O$ (O below N)

500 $S=^{\text{thiazole}}SN5=O$ (C-55 below)

Characteristics of the organism

11. We know now that the Fe^{+3} ion is in the blood (and within the organism)

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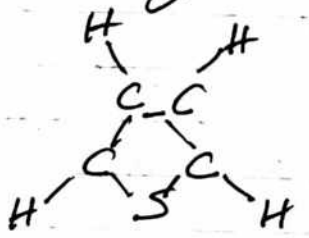
10. Molar absorbtivities from $n \rightarrow \pi^*$ transitions are relatively low, and range from 10 - 100 L/(mol · cm).

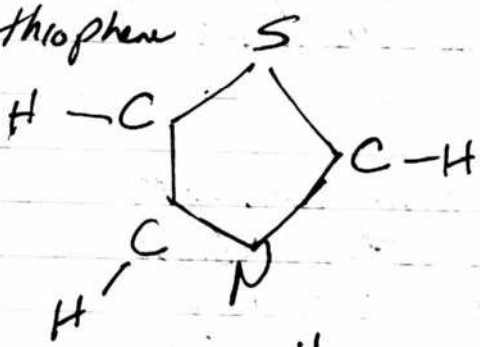
---

thiophase "55" =



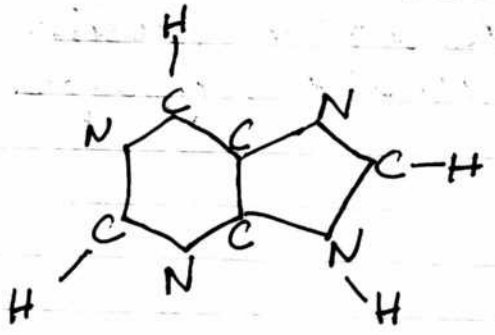
HONC

thiophene



sulfur - yellow
Carbon - black (or grey?)
white hydrogen.
blue - nitrogen

purine



If they do not label it as carbon

The Japanese book we found is for organic compounds, there is no reason whatsoever to assume it is organic. Surface evoline indicates that it is INORGANIC as $Fe(OH)_3$.

but Conjugation means alternating single double bonds

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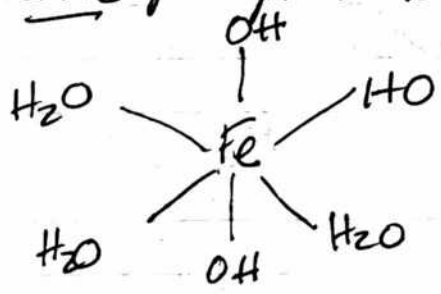
Ferric precipitate is $Fe(OH)_3$



It is ferric hydroxide we are looking at

'Unsaturated' in organic chemistry means that some of the carbons have double bonds (alkenes) or triple bonds (alkynes) and the carbons are not saturated w/ hydrogen bonds.

Examples are
- nitro
- nitroso
- azo
- azoxy
- carbonyl
- olefinic

Ok, How to Interpret

06/17/10

You are not properly identifying the higher risk individuals.
I cannot determine if in seven or below
One idea is, also age factored

X_i - Reference Hemoglobin (Age Factored)

[redacted]

100 no
99 no
60 no
65 no
56 no
54 no
54 no
~~46 no~~ 50 no
46 no

Larry C.

X_i - Avg Hemoglobin (Age Factored)

$\bar{X}$

[redacted]

100 no ① 75 no
44 no ② 72 no
42 no ④ 54 no
39 no ⑥ 46 no
35 no ⑤ 52 no
33 no ③ 66 no
26 no ⑦ 40 no
10 no ⑧ 33 no
7 no ⑨ 26 no

X_i - Reference Hemoglobin No Age Consideration

[redacted]

100 no
93 no
92 no
90 no
88 no
83 no
~~78 no~~ 81 no
~~77~~
81 no
76 no
77 no

X_i - Avg Hemoglobin No Age Consideration

[redacted]

100 no 89 no
41 no 66 no
41 no 70
31 no 62
26 no
25 no
16 no
10 no
8 no

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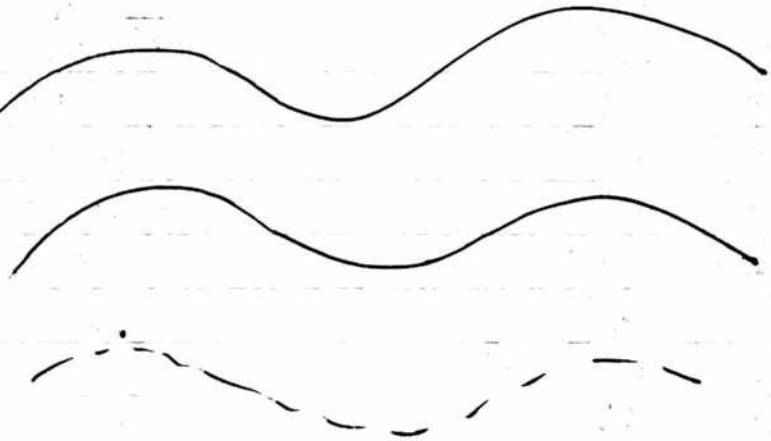
Age factored Combination results look fairly reasonable.

$$\frac{(X_i - \text{Ref H}) + (X_i - \text{Avg H})}{2}$$

$$= \frac{2X_i - \text{Ref H} - \text{Avg H}}{2}$$



where $X_i = \left(\frac{\frac{\Sigma V^2}{n}}{\text{Age}^{3/4}} \right)$

Ranking is:

Age		
60	[redacted]	15th
29		72nd
23		66th
58		54th
~50		52nd
66		46th
62		40th
56		33rd
70		26th

So yet characters highly favored homeowners and favor population and favor younger you are @ higher risk.

This model still seems the best

Qualitative	Quantitative	Model
1	2	1
2	3	2
3	4	3
4	5	4
5	6	5
6		

Something indeed has happened w sulfpha even though it is insoluble? Insoluble in HCl also. ???

Bactrim is a sulpha based drug. Some people are having life threatening reactions to its use. Be very careful w sulpha no matter what the results.

Page 234

We are seeing that what we are really does =

$$\frac{f(\sigma_1, \sigma_2)}{\text{age } P}$$

What if you added all the residuals?

Didn't we learn that 2 SDs corresponds to C

1z = 68% normal
1z = 50% my curve

n = 30 = how many %. –

Z	Area
0	Ø
.2	15.8
.5	38.3
1.0	68.3
1.5	86.6
2	95.4
2.5	98.8
3.0	99.7
4	99.9

Normal Prob $\tilde{=}$ $1.27 + 96x - 30.01x^2 + 3.08x^3$
$R = 99.9$

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Z Score evaluation from hemoglobin given

Use the following:

	X-Avg	(X-4)
85 no.	100 no.	92 no.
63 no.	75 no.	69 no.
41 no.	3 no.	11 no.
39 no.	9 no.	19 no.
37 no.	16 no.	24 no.
23 no.	57 no.	36 no.
23 no.	56 no.	36 no.
16 no.	69 no.	33 no.
10 no.	110 no.	28 no.

Questionable??

[redacted]

[redacted] are the closest get.

$* \left(\frac{X}{100}\right)^2$

Each image -

We may be having the same "issue" w/ urine as we are blood.

You do have a peak around 380 and the same dropoff @ 391.

We also have a spectrum of urine from a prenatal Verity (fluorescent spectrometry) and they have a peak near 430.

This may be showing the same type of shift.

Now we also took a lot of magnesium today (both magnesium oxide and magnesium sulphate) and a Rm test (NaOH or ammonia added) does show magnesium in the urine and that makes a lot of sense.

St. Judah urine does not show the Mg ion by ion testing. But has spectrum looks the same. This means the spectrum is not showing magnesium. It is showing something else. What? It may be a weak form of the Culture.

Ferrous (Fe^{+2}) Sulfate vs Ferric?

Ferric Sulfate + H_2O_2 IS Fenton's reaction.

$Fe^{2+} + H_2O_2 \Rightarrow Fe^{3+} + OH^- + HO^\circ$ This is perfect

but it reverses itself (actually it cycles!)
to
$Fe^{+3} + H_2O_2 \rightarrow Fe^{2+} + H^+ + HO_2^\circ$

It did, however, positively turn to yellow.
This is great, you are proving the Fe+3 ion appears to be in the culture growth in the presence of lye & heat. (oxidized) Fe +2 to Fe +3 means it loses an electron.

Spectrum of ferrous sulfate & ferric sulfate) is entirely different.

You are getting mixed messages now.
Ferric sulfate is not immediately turning green - why? It actually is turning yellow just & spectrum is same as ferric sulfate.
Why?
Does it turn green eventually?
It seems like it will have to.

We do know now that a dominant component of the culture is iron. We also know it does not become known until you have lye & heat.

X

More characteristics of the organism spectrum
Page 238

the list of knowledge increases

12 Chemical tests are available for Fe^{+2} and Fe^{+3} (very sensitive tests)

13. Fe^{+2} should be in the blood, not Fe^{+3}

14. Fe^{+3} solutions are acidic.

15 Spectrum has absorption flattens.

We found a source that sells a 0.12 oz solution of (1,10) 500 mg for $44.

We can buy 1 gram for $10. How much would this make?

Molecular wgt of (1,10) is 180.21 gms/mole
$C_{12}H_8N_2$

Molecular wgt of water is: 18.015 gm/mol

(1,10) is soluble in alcohol
only slightly soluble in water. 2960 mg/L

Isopropyl alcohol molecular wgt = 60.09 gms/mol

Fe+3 Causes 1. no binding to oxygen
4. T°est.mate - asymptomatic 2. produces free radicals
5. may be also occurring in the mitochondria 3 more acidic 6. It takes energy from our biological systems

% by mass = mass of solute / mass of solution

so a .12% of (1,10) means

~~LSA~~ ~~DF~~ 1%

100 gm ml =

.12 gms / 100 ml = .12% ok, so 1 gram is plenty.

Now 2.960 gm / liter will dissolve in water

so

2.960 gm / liter = .296 gms / 100 ml

and we only need to dissolve .12 gms so you can use water.

So we can use .2% without a problem.

.2 gms / 100 ml of water = .2% and this will dissolve.

Sodium Thiocyanate

10% weight / Volume solution is one that is solid. This means

10 gms / 100 ml fine, we are getting 100 gms

7. Antagonist Factor's reaction also produces a free radical

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We have

element	vs	compound
atom	vs	molecule

what exactly is the difference?

An element is composed of atoms of the same type.
An element (a chunk of sulphur) for instance is divisible.
An atom is not.
Molecule - a fundamental unit of a compound

The only change that occurs in a chemical reaction is the arrangement of the atoms.

We have what is called an Ionic Compound

MgO (Mg^{+2} O^{-2}) = neutral it is an ionic compound

But they actually are ions that are bound together

There are <u>lots</u> of them.

$Fe^{+2}SO_4$ ferrous sulfate

$Fe^{+3}(OH)_3$ ferric hydroxide

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Heme B is $C_{34}H_{32}O_4N_4Fe^{+2}$ state

We are looking for something that will form between C – Fe^{+3}
- has a double bond with normally N, O, or S

alkene (double bonds)
alkyne (triple bonds)

We expect a ferric ion combined w/ an alkene or alkyne

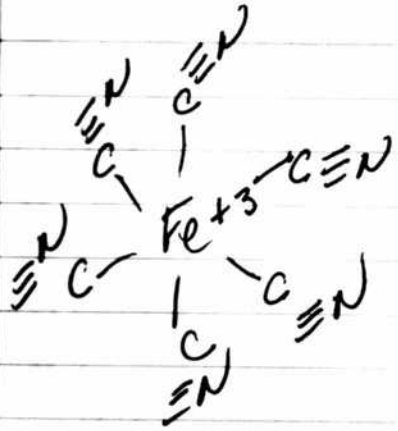
It is a hetroatom!!! NOT C=C C≡C

eg hexacyanides $[M(CN)_6]^{3-}$ and C≡N is triple bonded to Nitrogen

M = Ti, V, Cr, Mn, Fe, Co

Ferric hexacyanide is $C_6H_3FeN_6$
also called ferricyanide

formula is $[Fe(CN)_6]^{3-}$ much less toxic

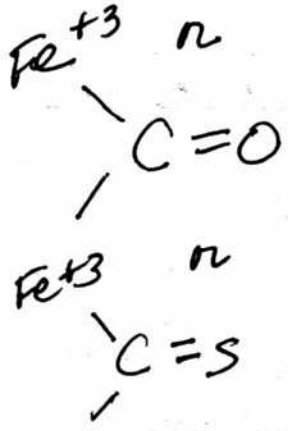


alkenes must have C=C

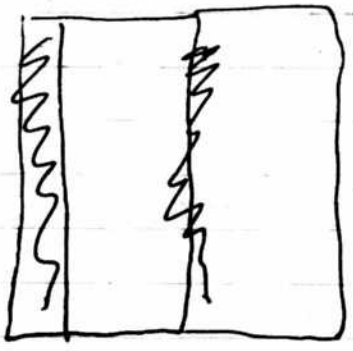
Not alkene or alkyne!

We need

$Fe^{+3} - C \equiv N$



Now Heme already has (N)



Mike GB
Talk to GO

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$\frac{\Sigma V^2}{n \text{ individuals}}$ = Avg V^2 per individual

Good Bad Middle

[redacted]

Ok, here is a problem. If your translation is off it will give you a big deviation.

I think we need to subtract the average.

Avg rating of 442.5 peak to 397 peak is = 1.06 is $\frac{2.27}{2.14}$

[redacted]

442.5/397 =	Age	ΔAvg
1.09	58	+.03
1.06	56 ✓	0.00
1.202	62	+.14
1.202	29	+.14
1.011	60 ✓	−.05
1.154	66	+.09
1.00	23 ✓	−.06
1.17	50	+.11
1.05	70 ✓	−.01
1.01	68 ✓	−.05
1.12	60	+.06

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I do not think this test is a good test. ie, the ratio test.

Subtract out the reference spectrum and look at they are!

Remove the trend from both curves gives you a very nice Comparison.

Wavelength
Reference
Average
[redacted] (close to average)
[redacted] (far from average)

Deviation from average approach leads to strongest candidates

80-100 Next set is 60-79 Next set is 40-59

[redacted]

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This is now looking very reasonable

$$C = \frac{1}{\Delta x_i} \tan\left(\frac{Pr}{\left(\frac{200}{\pi}\right)}\right) \qquad \Delta x_i = \frac{1}{C} \tan\left(\frac{Pr}{\left(\frac{200}{\pi}\right)}\right)$$

$$Pr = \left(\frac{200}{\pi}\right) \tan^{-1}(C \cdot \Delta x)$$

Through our probability model, much simpler

Non Age Scaled

70 ~~69~~ - 90+ | ~~60~~ - ~~70~~ 69 | < 60

[redacted]

Age Scaled

80 - 90+ | 75 - 79 | < 75

23
29
60
66

[redacted]

58, 62
50
60

[redacted]

68
70
45

[redacted]

Current Ranking (Age Scaled)

[redacted]

90
86
84
83
~~79~~
~~79~~
~~79~~

[redacted]

80
79
78

[redacted]

~~76~~ 11
~~75~~ 13
~~74~~ 70
70

[redacted]

Non Age Scaled

[redacted]

90%
89%
72%
71%
~~67~~%
~~60~~%
60%

[redacted]

59
53
C
53
45

Theory:

Highest variation relative to the norm of the population relative to their age.

Prob $\frac{f(X_i - \bar{X})}{Age^P}$

Now it is possible that the culture contains cytochrome, but not a active they. Cytochrom basically has the same specificity as longlater since they are essentially the same

Cytochrom oxidized is different from cytochrome reduced.

Oxidized mean lose electron.

Looks like it shifts from about 414 to 405 from reduced to oxidized.

Also the peaks in the 560 region demonstrate oxidation

Jun 21

We have a difference in the culture spectrum w/ the passage of time.

The peak @ 418 is dominant.

There is nothing showing up @ 560nm

Actually it still is essentially the same

There is no direct evidence that the Culture Conforms to Cytochrome but it also has not been entirely disproved.

The obvious thing to do is to test for Iron Fe+2 or Fe+3 in the Culture.

but it is being oxidized by the lye & heat.

But the blood shows the same Fe+3 spectrum

The result indeed indicate that Iron is being oxidized to a state of Fe+2 to Fe+3.

Fe+2 Can bind oxygen Fe+3 Cannot.

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You all hot on the trail.

1. How do you make green $Fe^{+2}SO_4$?

2. Analytically combine Fe^{+3} spectrum with yellow hemoglobin to create measured blood.

3. Reaction of Fe^{+2} w/ H_2O_2 to give Fe^{+3}?

4. Study redox reactions!

$FeNH_4(SO_4)_2 \cdot 12H_2O$ Ferric Ammonium Sulfate

It also works!
397 nm, sharp rise, gradual decline.

You now have two ways of showing the ferric ion in solution

Ok, what is happening is the ferric ion will give you the first peak @ 397 but it does not give you the second peak @ 446 nm nm? What causes the second peak?

Clearly a part of the culture is the ferric ion but there is something else much stronger @ 446 nm. Where is the comparison.

We see that the concentration of "3" in the culture matches the average blood spectrum almost exactly. This means you can calculate the amount of the culture in the blood.

Next we are taking .75 * our concentration of blood @ 11 mg/liter

---

What we learn is that the culture @ concentration "3" matches essentially exactly the average blood spectrum from 340 to 450 nm nm.

Ok, you did pick up the second peak w/ sufficient concentration of ferric ammonium sulfate!

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1. Take Reference
2. Ferric Concentrate
3. Blood Average

What we see is that from 340 - 510 nm

ferric ion explains almost exactly the spectrum that occurs in measured blood (all curves detrended).

On the right side (520+) there is almost no influence. You truly have a mixture model that you could solve for.

AVG Blood = Ferric Ion + Reference Hemoglobin

Least square model will give a very good solution here.

Set up the Model.. n=15

$A_{MS} = C_1 \cdot Fe^{+3} + C_2 HbO_2$

α RED β BLACK Δ Model BLUE

V+	Fe+3 Red (β)	HbO2 Black (β)			Means (Blue) (Δ)	V's
340	0	0	C_1	=0	0	.
364	.55	.49	C_2	.64	.10	-.54
397	1.22	.74		1.13	1.28	.15
401	.63	.86		1.00	.66	-.34
416	1.05	1.23	This pt is distorting the model	1.48	1.06	-.42
442	1.29	.66		1.09	1.49	.40
452	.85	.52		.79	.95	.16
477	.39	.39		.50	.65	.15
509	.47	.19		.35	.47	.12
542	.12	1.13		1.02	1.42	.40
560	.07	1.05		.93	.90	-.03
577	.01	1.42		1.20	1.42	.20
600	.05	.36		.33	.06	-.27
645	-.07	-.24		-.23	-.01	.22
700	0	0		-.007	0	.007

$\sigma = .11$ $C_1 = .401$

$\sigma = .09$ $C_2 = .86$

Σ_{xx} (Not this)

$\sigma = .151$

$\sigma = .108$

looks very good

$\frac{\Sigma V^2}{n} = .082$

$\sigma = .29$ Not bad

All curves detrended

$\frac{1}{n}\Sigma_{xx} = .082 \begin{bmatrix} .151 \\ .108 \end{bmatrix} = \begin{matrix} .012 \\ .009 \end{matrix}$ $\sqrt{.012} = .11 = \sigma$ $\sqrt{.009} = .09 = \sigma$

These are very good numbers!

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Lots of Questions

All kinds of questions:

done 1. What is spectrum of blood in alcohol vs water?

No 2. Is there any possibility that red blood cells oxidize in water to create Fe^{+3}? It should be HBO_2

done 3. Need concentration graph of Fe^{+3}
$FeCl$ } 2 kinds
$FeNH_4SO_4$ } are they the same

4. [redacted] questions

→ For #2. Blood in Alcohol precipitates
You can still see the peaks @ 397 & 448 however.
The precipitate are the proteins in blood.
The Color of blood changes to a pale solution

5. Mixing Fe^{+3} with blood causes
What Color?

6. Shelf life of blood

$\frac{.97 \text{ gms } Fe^{+3}Cl_3}{4 \text{ ml } H_2O}$

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Calibrating Eyedropper Again!

It all dissolved.

Interesting. I have a 3rd peak.

Calibrate eyedropper again.

$\frac{68 \text{ drops}}{4 \text{ ml}} = \frac{1}{X}$ $\quad X = .059$ ml or 0.06

1 drop = .06 ml

So take out 1 ml = 17 drops

$\frac{.97 \text{ gms}}{4 \text{ ml}} = \frac{X}{1}$ $\quad X = .2425$

So our solutions are

.2425 gms/ml

.1212 gms/ml

.0606 gms/ml

Now use $\frac{1.02 \text{ gms}}{30 \text{ ml}}$

1 ml = 16.1 drops

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We have 1.02 gms / 30 ml in tube

Get 2 ml in each test tube

		Δ
(1)	35	Ø
(2)	35 − .33(35) = 23.45 (23)	12
(3)	23.45 − .33(23.45) = 15.64	19
(4)	15.64 − .33(15.64) = 10.43	25
(5)	10.43 − .75(10.43) = 2.6 = 3	32

$\frac{1.029 m}{30 ml} = \frac{x}{1}$ $x = .034$ gms/ml = 34 mg/ml

		A
(1)	34 mg/ml	1.745
	23 mg/ml	1.770
	15 mg/ml	1.699
	10 mg/ml	1.602
	2.5 mg/ml	.478

This curve is not @ all linear.
Not even close

It looks to me like it is way too concentrated

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Ferric Chloride
Calibration Graph

Sounds like we should be using from 35

#	Drops	Conc	A	
2	1	2 mg/ml	34	But this is 2 ml!!!
3	2	4	33	This test was actually very sensitive
4	3	6	32	
5	~~5~~	10	30	
	~~7~~	~~14~~	.	
6	10	20	20	

OK. Now you have a good graph.
You had the concentration way too high by a factor of 3 to 1.
~~You should only~~

Concentration of original solution was ~ 34 mg/ml
Each drop is .06 ml

		A	Date
1 drop in 2 ml	= .06(34)/2 ml =	1 mg/ml	.141
2	(2)(.06)(34)/2 =	2 mg/ml	.297
3		3 mg/ml	.664
5		5 mg/ml	1.022
10		10 mg/ml	1.658

$A = .0853 \cdot (2 \cdot Conc)$

So:

$A = .1706 \cdot$ Concentration of $FeCl_3$

or Concentration $FeCl_3 = \frac{A}{.1706}$

This looks reasonable.

Now what about $FeNH_4SO_4$? Same?

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Here is a question.

If a Compound is so much mass of $FeCl_3 \cdot 6H_2O$

How much of the mass is actually Iron?

Molar mass is 270.295 gms/mole

	Mass %	Therefore
H	4.45	
O	35.52	
Cl	39.35	
Fe	20.67	

* Concentration of Iron Iron $= \frac{A}{.1706}(.21) = A * 1.23$

So. ~~Concentration of Iron Ion $^{+3}$~~ $= \frac{A}{.1706(\text{[illegible]})} = \frac{A * 1.23}{\text{[illegible]}}$

Example of $A = 1.65B$

Concentration of Iron Ion $^{+3}$ is: $(1.23)\ 1.65B = 2.04$ mg/ml

of the actual iron ion.

There is a very small amount

We know in full blood we have about 150 mg of hemoglobin per ml.

We are putting about 2 drops of blood in a ml of water. So

$2(.06\text{ml})(150\text{mg/ml}) \cong 18\ \frac{\text{mg}}{\text{ml}}$

We know we are actually using about $11\ \frac{\text{mg}}{\text{ml}}$

So how much of this is iron?
What is the formula for hemoglobin?

$C_{3032}H_{4816}O_{872}N_{780}S_8Fe_4$

$C_{2952}H_{4664}N_{812}O_{832}S_8Fe_4$

From sources, Molar Mass of hemoglobin is $65,700\ \frac{\text{gms}}{\text{mole}}$

~~hemoglobin~~ is 223.4 gms/mole

Iron is $4(55.8) = 223.4$ gms/~~hemoglobin molecule~~.

hemoglobin

$\frac{150\text{mg}}{\text{ml}} = \frac{150\text{ mg}}{65,700\text{ gms}} = 2.28E-6\ \frac{\text{moles Hemoglobin in 1 ml}}{\text{ml}}$

The Iron Mass is .34% so $.0034\ \frac{(150\text{mg})}{\text{ml}}$
To Hemoglobin Mass

$= .51\ \frac{\text{mg Fe}}{\text{ml}}$

but we are only using $11\ \frac{\text{mg}}{\text{ml}}$

Hemoglobin is .34% mass of iron relative to the mass of the hemoglobin.

Therefore if we are using a concentration of approx $* \frac{11 mg}{ml}$

$= 37 ug/ml$

very small

We have $.0034 \left(\frac{11 mg}{ml}\right) = .037 \frac{mg}{ml}$ of Fe

You might have enough information

$A = abc_1 + abc_2$

now to start formulating concentration?

You need to work on $FeNH_4SO_4$ (calibration curve to see if you get the same amount of work)

Ferric Chloride result:

$Fe^{+3} \cong \frac{A}{1.23}$ in mg/dl

This work is showing a very high level of sensitivity.

Ferric Ammonium Sulfate Calibration Curve Fe^{+3}

$Fe\,NH_4(SO_4)_2 \cdot 12H_2O$ Method 2

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Now lets do the same for $Fe\,NH_4\,SO_4$:

2.23 gms in 30 ml 35 drops for 2 ml

Drops	Δ	Σ	Conc (in 2 ml)
5	30	35	11 mg/ml
10	25	35	22 mg/ml
15	20	35	33
20	15	35	44
25	10	35	55

$\frac{2.23\text{ gms}}{30\text{ ml}} = \frac{X}{1\text{ ml}}$

$X = .0749\text{ gms} = 74\text{ mg/ml}$

Conc 5(.06) = 30 ml $\left(\frac{74\text{ mg}}{\text{ml}}\right)$ tm = 22.2 mg

and $\frac{22.2\text{ mg}}{2\text{ ml}} = \frac{11.1\text{ mg}}{\text{ml}}$ It is way too high again

Drops	Δ	Σ	
.1	34	35	2.2
2	33	35	4.4
3	32	35	6.7
5	30	35	11.1
10	25	35	22

Abs = .0597 × Conc in mg/ml

or Conc. in mg/ml = $\frac{A}{.0597}$

The FeCl was $\frac{A}{.1706}$

Molecule Mass = 524.2 Fe^{+3} % = 10.65%

so Conc Fe^{+3} in $\frac{mg}{ml} = \frac{A}{.0597}(.1065) = \frac{A}{} \cdot 1.784$

The average of both solutions is

Conc Fe^{3+} = 1.50 · A in mg/ml

And the two solutions are C_{one} = 1.23 · A
C_{two} = 1.78 A

This is not unreasonable.

We seem to have a method now of determining the concentration of the Fe^{+3} ion in a solution.

We should also know what the concentration of hemoglobin. How can we determine the concentration of each in a mixture?

We know $A = a_1 b c_1 + a_2 b c_2$

a is absorptivity, a coefficient. $\frac{\text{liter}}{\text{gm} \cdot \text{cm}}$
b is pathlength in cm
C is Concentration in gms/liter
but mg/ml is the same

$\frac{\text{liters}}{\text{gm} \cdot \text{cm}} \cdot \text{cm} \cdot \frac{\text{gm}}{\text{liter}}$ so A is only a number!

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Now we have already created a model:

@ $A = .407\,Fe^{+3} + .86\,HbO_2$
(b.c) a (b.c) a

391nm where Fe^{+3} is the measured absorbance of Fe^{+3}
576nm HbO_2 is the reference hemoglobin

The coefficients have a very low standard error, ≅ 0.1

To determine concentration he sets up
(path length. Concentration) (path length. Concentration

391 $1.13 = \underline{1.22}\ .407 + \underline{1.42}\ (.86)$

Iron reference Concentration — HbO_2 ref Concentration

$\lambda = 391$ — $\lambda = 576$

(No influence from cell thickness)

modest influence for hemoglobin there.

The Iron reference Concentration is $1.50 \cdot A = 1.50(1.22)$
The hemoglobin Concentration is 11.0 mg/ml =

$1.13 = \frac{1.22}{1.50(1.22)}(.407) + 1.42(.86)$ (11 mg/ml)

$1.13 \overset{?}{=} .27 + .11$

$1.13 \overset{?}{=} .38$ Nope!

391:

$1.13 = .407\,C_x + .86$

Cr = reference concentration in gms/liter or mg/ml

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391 ~~576~~ 391

391 $1.13 = \frac{1.22}{Cr(Fe^{+3})} \cdot Cx + \frac{.01}{Cr(HBO_2)} Cy$

576 $1.20 = \frac{.74}{Cr(HBO_2)_{576}} \cdot Cx + \frac{1.42}{Cr(HBO_2)_{576}} Cy$

template

$$A_{391} = \frac{a_1@391}{Cr(mixture\,1)^{Fe^{+3}}} \cdot Cx + \frac{a_2@391}{Cr(mixture\,2)^{HBO_2}} \cdot Cy$$

$$A_{576} = \frac{a_1@576}{Cr(mixture\,1)^{Fe^{+3}}} \cdot Cx + \frac{a_2@576}{Cr(mixture\,2)^{HBO_2}} \cdot Cy$$

So for us:

@391 $1.13 = \frac{1.22}{Cr(mixture\,1)} \cdot Cx + \frac{.74}{Cr(mixture\,2)} \cdot Cy$

@576 $1.20 = \frac{.01}{Cr(mixture\,1)} \cdot Cx + \frac{1.42}{Cr(mixture\,2)} \cdot Cy$

any frequency $A = .407 \cdot a_1 + .86 \cdot a_2$

Brilliant. You have solved it.

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Therefore we know that @ 391nm

$.407 \cdot a_1 = \frac{1.22\ C_x}{C_{r(mixture)}}$

We know the concentrations of each of the stock solutions but we do not know how much of each we mixed together to create the final result. This is what we have solved for.

but we know that $a_1 = 1.22$ so

any λ @ 391 $.407 = \frac{C_x}{C_{r(Mixture\ 1)}}$ and $.86 \cdot a_2 = \frac{.74\ C_y}{C_{r(mixture\ 2)}}$

any λ @ 391 but we know that $a_2 = .74$ so

$.86 = \frac{C_y}{C_{r(Mixture\ 2)}}$

but also @ 576:

@ 576 Testing:

$1.13 \stackrel{?}{=} 1.22(.407) + .74(.86)$

$1.13 \stackrel{?}{=} 1.13$ yes, very good.

only can be determined @ 576

only can be determined @ 391

But we know that $C_{(Mixture\ 2)} \stackrel{\sim}{=} 11.0$ mg/ml

and that $C_{(Mixture\ 1)} \stackrel{\sim}{=} 1.5(1.22) = 1.83$ mg/ml $= 1.5\,A_{(Fe^{+3})}$] Fix ED

so @ 391: $C_x = .407 / \left(\frac{1.83\ mg}{ml}\right) = 0.74$ mg/ml

Say the total mass of HbO2 in the culture grown in $C_y = .86(11.0\ mg/ml) = 9.46$ mg/ml

This is an answer!!!!!

Lets perform a similar calculation
@ ~~547nm~~ 576nm

$1.20 \stackrel{?}{=} .01(.407) + 1.42(.86)$

$= 1.22$ Very Good

~~@ 576nm:~~

~~$Cx = .401/(1.5(.01)) +$~~

So our end conclusion here is that roughly 7% of the mass of the hemoglobin has been converted to an Fe^{+3} state.

This is the condition (oxidized hemoglobin) called Methemoglobinemia.

10-15% would cause bluish skin.

Three methods,

1. My method
2. Cola test
3. Medical test

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This could be developed as an alternate project.

Now the question is,

How would you go about this in an individual basis?

1. Test for oxidation of the blood.
2. Need methemo model.
   Methemoglobin
3. Spectrum of methemoglobin – looks right

I have 246

Calc Value ~~Methemo~~ = $-1.25x + 210.3$

$-1.25x =$ Calc Value $- 210.3$

$x = \dfrac{\text{Calc Value} - 210.3}{-1.25}$

Scan higher than 210.3 says no problem

Sodium Nitrite can be used to induce Methemoglobin

Scanners can vary.

My method looks much more accurate.
Develop an individual procedure?
Or just refer to medical tests.

[redacted]

Now you need to define what you actually have accomplished & how you have done it.

First of, we have a "features" observation, and that is that the spectrum of the culture is essentially identical to that of Fe+3.

Two other parts are needed early in the game; a reference hemoglobin spectrum (ie a calibration graph) for hemoglobin and in addition a reference (calibration graph) for the concentration of Fe+3.

Another incredible observation is that the spectrum of hemoglobin as IT IS BEING MEASURED shows itself to be a likely combination of the reference hemoglobin and the Fe+3 spectrum.

primary

You now solve for the combined spectrum in a least squares sense and arrive at an excellent model which does indeed show that measured blood (average of 11 individuals) can indeed be created as a linear combination of the spectrums of reference hemoglobin and Fe+3.

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We now solve a system of equations according to Beers Law.

Now Beers Laws

$A_1 = a_1 b C_1 + a_2 b C_2$

Now let's go over units.

$a = \frac{\text{liter}}{\text{gm} \cdot \text{cm}}$ a is absorbtivity coefficient.

$b = \text{cm}$ b = path length.

$C = \frac{\text{gm}}{\text{liter}}$ C = Concentration

so $\frac{\text{liter}}{\text{gm} \cdot \text{cm}} \cdot \text{cm} \cdot \frac{\text{gm}}{\text{liter}} = A$, a number as it should be.

Now in our book (Thomas) he uses the form

λ_1 $A_1 = \frac{\text{Measured absorbance}_{\lambda_1, \text{Mix } 1}}{\text{Reference Concentration}_{\text{Mix}_1}} C_1 + \frac{\text{Measured Absorbance}_{\lambda_1, \text{Mix } 2}}{\text{Reference Concentration}_{\text{Mix}_2}} \cdot C_2$

λ_2 $A_2 = \frac{\text{Measured absorbance}_{\lambda_2 \text{ Mix}_1}}{\text{Reference Concentration}_{\text{Mix}_1}} C_1 + \frac{\text{Measured Absorbance}_{\lambda_2, \text{Mix}_2}}{\text{Reference Concentration}_2} \cdot C_2$

Now, how does the set equate to the above units?

We see that this form is exactly what I solved for in my model.

This means that there is an alternative formulation of Beers Law in a much simpler form for a mixture ie

$$A_M = C_1 \cdot A_1 + C_2 A_2$$

where C_1 & C_2 are coefficients! not Concentrations! and
A_1 & A_2 are measured absorbances.

and that C_1 & C_2 coefficients are actually equal to

$$C_1 = \frac{C_x}{C_r \text{ (Mixture 1)}} \qquad \begin{array}{l} C_x = \text{Concentration of } x \\ C_r = \text{ref Concentration of } x \end{array}$$

$$C_2 = \frac{C_y}{C_r \text{ (Mixture 2)}} \qquad \begin{array}{l} C_y = \text{Concentration of } y \\ C_{r_2} = \text{ref Concentration of } y \end{array}$$

So what we are really saying is that

$$A_M = \left(\frac{C_x}{C_{r_1}}\right) A_1 + \left(\frac{C_y}{C_{r_2}}\right) A_2$$

and so with this method what you are really solving for is the ratio of the actual Concentration to the reference Concentration. These are the unknown of the system.

Now, where do we find this alternative formulation of Beer's law in a ratio sense?

Now, where did this formulation come from? And how does it relate to the original formulation $A = a_1 b_1 c_1 + a_2 b_2 c_2$

Those formulations is all based upon ratios of concentrations.

When we solve our problem we get coefficients. These coefficients actually are ratios of concentrations, not concentration in themselves.

so we get a number, call it b_1 or b_2

$b_1 = \frac{C_x}{C_{r_1}}$ is an actually problem to solve after we formulate our model based upon measured absorbances above.

So $C_x = b_1 \cdot C_{r_1}$ and C_{r_1} is the reference concentration of component #1.

(our coefficient from model!)

↑ from calibration curve

This means you must know the parts of the whole under a Calibration Curve before you can solve the problem.

We are very lucky to have solved this intuitively as they of Fe+3 else you would have been stuck.

Beers law is only valid for low concentrations!

The slope of the calibration graph is the molar absorbtivity ϵ

How about that?

$A = \epsilon \cdot c \cdot l$ we set $l = 1$?

$\frac{dA}{dc} = \epsilon$

So molar absorbtivity must be defined as $\frac{dA}{dc}$

ie the change in absorbance w.r.t. to change in concentration

and it is a constant in our case (ie the slope of a line).

Ok I found my answer there is indeed an alternative formulation to Beer Laws:

" The ratio of the concentrations is proportional to the ratio of absorbances ".

So $\frac{C_1}{C_2} = \frac{A_1}{A_2}$

Now notice our form of

$A_M = \left(\frac{C_x}{C_{r_1}}\right) A_1 + \left(\frac{C_4}{C_{r_2}}\right) A_2$

and reduce it to a single component

$A_M = \left(\frac{C_x}{C_{r_1}}\right) A_1$ Notice how this looks like a ratio???

$\frac{A_M}{A_1} = \frac{C_x}{C_{r_1}}$ and $A_{MIXTURE}$ in a single component solution simply becomes A_x

So

$\frac{A_x}{A_1} = \frac{C_x}{C_{r_1}}$ and A_1 here actually means the absorbance of the reference solution.

This form of Beer's law is much more intuitive

$\frac{C_1}{C_2} = \frac{A_1}{A_2}$ very simple & practical

at a single wavelength & same path length

actual law is $\frac{C_1}{C_2} = k \frac{A_1}{A_2}$ but k must equal 1 if wavelength & path length are same for both.

We can now see that having accurate reference concentrations of the components is critical or everything is wrong.

So now we reexamine how we arrived @ these values.

Hemoglobin was done theoretically

Fe^{+3} was done by direct calibration graph using two different ferric salts and averaging the results. We isolated the ferric ion by molecular mass composition on % terms. Your results came out quite well.

Ultimately then, you were able to solve for C_x & C_y in the mixture (the mixture is measured blood)

C_x = Concentration of ferric ion in average measured blood

C_y = Concentration of hemoglobin in measured blood.

You receive numbers of

@391nm using Conc. of $Fe^{+3} \cong 1.5 \cdot A$ in $\frac{mg}{ml}$ @ 391nm,

λ=516 Conc of reference hemoglobin
is determined analytically using the
average.
The probably has an error in it.
But the error should be very small
because the Fe^{+3} has very low absorbance
@ 516 nm.

So we choose avg reference hemoglobin
Concentration is ~ 11.0 mg/ml

So we arrive @ % by

C_x $1.5(1.22) = 1.83$ mg/ml of Fe^{+3}
C_y $\cong$ 11.0 mg/ml of reference hemoglobin.

391 $.401 = \frac{C_x}{C_{r_x}}$ $C_x = .401 \cdot C_{r_x}$

You solved for ratio of absorbances

$C_{r_x} = 1.5/1.22 = 1.83$ mg/ml as meas ~~(1.83)(.41)=~~ $1.83(.401) = .74$ mg/ml

516 $.86 = \frac{C_y}{C_{r_y}}$ $C_y = .86 C_{r_y} = .86(11) = 9.46 \frac{mg}{ml}$

So

$\left(\frac{1.83}{1.83 + 9.46}\right) = 7.2\%$ methemoglobin estimate.

.74 .74

Lets think about our unanswered questions:

but it will not bind up so must be happening

1. Molecular model of methemoglobin? Itony charges Fe^{+2} to Fe^{+3}
2. Green iron sulfate Fe^{+2}?
3. How does the organism actually change it from a Fe^{+2} state to a Fe^{+3} state?
   ie What causes the oxidation?

   Does Flatorn reaction produce Fe^{+3}?

   Yes, this is exactly what Ferrous reductase is.

In the test tube:
Ferrous Sulfate $Fe^{+2} + H_2O_2 \rightarrow Fe^{+3}$

4. How would ya determine the MH (Methemoglobin) blood level of an individual?
5. Mixing Fe^{+3} w/ blood cause what color?

11 Cytochromes are a group of heme containing proteins located in the mitochondrea.

This means the problem could be taking place in the mitochondria.

Mitochondria is an organelle found in large numbers in most cells in which the biochemical processes of respiration and energy production occur.

They are the cells power producers.
ATP is the cells energy currency
Mitochondria produce ATP

Mitochondria have DNA
Size 1-3 microns (maybe 1-10 microns)

Ferrous sulphate is found in Lawn Moss killer
$FeSO_4 \cdot 7H_2O$

Making a $FeSO_4$ solution water

40 ml H_2O
6 tablets @ 325 mg each.

$= \frac{1950 \text{ mg}}{40 \text{ ml } H_2O}$

$1 \text{ Mole Solute} = \frac{278.02 \text{ gms}}{1 \text{ mol}} = \frac{1950 \text{ mg}}{X}$

$= .007$ molar solution.

1 mole solute $= \frac{278.02 \text{ gms}}{1000 \text{ ml}}$ but have $\frac{1.95 \text{ gms}}{40 \text{ ml}}$

We would have 11.12 gms in 40 ml for a 1 molar solution

$\left(\frac{40}{1000}\right)(278.02) = 11.12 \text{ gms}$

but we have 1.95 so we have a

$\frac{1.95}{11.12} = .175$ Molar Solution

We get 30 gms in our bottle

Lets Chang to 18 tablets in $\frac{60}{50}$ ml of water.

18 tablets, 60 ml of water

Fe(325 mg) = 5.85 gms

$$\frac{278.02 \text{ gms}}{1 \text{ mol}} = \frac{278.02 \text{ gms}}{1000 \text{ ml}} \quad \frac{X}{60 \text{ ml}}$$

X = 16.681 gms for 1 molar solution
but we have
$\frac{5.85}{16.681}$ = .35M Solution $FeSO_4$
if all dissolved.

Fe+3 Fe^{+3} has an electron configuration of $Ar\ 3d^5$

Fe Fe has an electron configuration of $Ar\ 3d^6 4s^2$

Fe+2 Fe^{+2} has an electron conf is of $Ar\ 3d^6$

Or, pull one more off and it is $3d^5$ makes sense

Sal Khan
An ionic bond is not a covalent bond. It is not sharing anything. The bond is based upon Coulomb forces.

Covalent bonds share electrons.

So what type of bond it is is really important!

Ionic, Covalent, Metallic Bonds, Polar
How do you know what type is likely?!
It is not always clear - they overlap

Amazing statements:

An ionic bond is not a molecule.
There is amazing.
Ionic bonds are an aggregate
not a molecule!
Ionic bonds exchange electrons
Covalent bonds share electrons

Electronegativity is a measure of
"how much" you want electrons.

It is the difference of electronegativity
that matters.

So what exactly is a molecule?

Pauling says a molecule is a group
of atoms bonded to ~~one~~ one
another.

(He does not say how - seems to me
that salt qualifies)

Looks like there is some gray area, some
say yes, some say no.

Looks like the final verdict is no.

Wikipedia molecule says

salts & metals are composed of
Chemically bonded atoms or ions
but are not made up of discrete molecules.

Alex says that molecules are held
together by Covalent bonds
(same as Moore said).

So in the end Moore seems to be correct.
There is all very confusing.

Free radicals contain an odd number
of electrons. There are both uncharged
radicals & radical ions. They are all highly reactive
In theme, we have F^{+2} bonding w/ Nitrogen

Fe = 1.8 3.0 - 1.0 = 1.2 = Pola-Covalent
N = 3.0

From answer.com

Free radicals are highly reactive because they
are missing and electron. They will take
an electron any way they can get it.

Ok we found the answer, ie
What is the effect of
oxidized iron in the blood

1. It can no longer bind to oxygen
2. It produces a free oxygen radical

Fe^{2+} → goes to Fe^{3+}

HbO_2 goes to Hb + O_2^-
Fe^{2+} → methemoglobin Fe^{3+}

This is the critical reaction. ← There is an oxygen free radical

This is from a PHD in biochemistry Dr PK Joseph

They react w/ DNA + RNA + "Wreck havoc in the living system"

From over the Humble MHS Site:
In living things, including parasites, iron is a necessary cofactor for many enzymes.

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Na_2O (+2 · +6 −2?) CaO (+2 −2?)

Elements in Group 1A, 2A 3A give up 1, 2, or 3

Elements in Group 5, 6, 7 accept 3, 2, 1

Metals give up
Non metals accept

Metals ~~accept~~ give electron
Non Metals accept

Na_2O (+2 −2) ok

GaN (+3 −3) ok

CaO_2 (+2 −2 = −4) ok – Not ok, why? ok $^{-2} \neq -4$

Li_3N (+3 −3) ok

AlF_3 (+3 −3) ok

$MgCl_2$ & I_2 Mixture Two Cy-S's

Ionic Covalent

Dissolves: also dissolves

The question is as how to get separated? The answer was to heat it up. I_2 was molecular covalent & must be more volatile.

H_2O_2 is naturally produced in organisms as a by product of oxidative metabolism

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We still have an important question

How does the organism oxidize the iron in the blood.

We know the organism proliferates in the environment of

$Fe^{2+} + H_2O_2 \rightarrow Fe^{3+} + OH + OH^-$

ferrous iron — hydroxy radical — hydroxide ion

The organism does well here.

We know the organism grows well in this environment.

If there was peroxide in the blood it would take care of it.

Hydrogen peroxide is formed in the thyroid, gut & lungs

Ok, we have a source that says white blood cells make hydrogen peroxide (So it is trying to solve a problem but it ends up causing a problem)!

!!!

Just Google hydrogen peroxide

Second reference says white blood cells produce H_2O_2

Peroxisomes found in almost all cells. They produce H_2O_2.

So the proposed sequence is.

1. Fe^{2+} & H_2O_2 exist in the body
   Some Fe^{3+} is bound to occur
2. The organism flourishes in this environment
   It also flourishes in a blood environment
3. The organism appears to feed on Fe^{3+} for sure.
   Maybe it feeds on Fe^{2+} also? Don't know
4. Appears to convert Fe^{2+} to Fe^{3+}
   upon the blood as discovered by
   the spleen of blood
5. Spleen of blood matches Fe^{3+} and
   a small combination of Fe^{3+} + hemoglobin
6. Fe^{3+} prevents oxygen from binding
   leads to condition of methemoglobinemia
7. Produces an O_2^- radical
8. Serum consequences, MH of 7th.
   Calculated
9. Proto Fe^{+3} creates a more acidic environment
10. May also be occurring in the mitochondria.

RMSS Star Party

We have a lot of good tools at our disposal even though we are in Clayfield.

1 Chemlab - purchased - lab simulations including redox. (great periodic table & molecular viewer)

Redox 2 Chemical predicta - redox reactions in detail

Redox 3 Chemix - all around tool
has molecular calculator
also redox reactions complete library
very good periodic table w/ oxidation states, electronegativity etc
least squares, solubility chart
lots of good things, a chemical balance!
eg $FeSO_4^{+2}$ is soluble in water
$Fe_2^{+3}(SO_4)$ is not.
Ferric Chloride & Ferric Nitrate are soluble in water, we now have both forms.

Redox 4. ChemTool Box
Also redox reactions under solutions
They are all listed in reduction form.

There tools can be used to answer the questions like does $Fe^{+2}SO_4$ oxidize in water?
vs peroxide

from Chemtoolbox

$FeSO_4 + H_2O \rightarrow ?$

$FeSO_4 + H_2O_2 \rightarrow ?$

$Fe^{2+} + 2e^- \rightarrow Fe$ -.447
$Fe^{3+} + e^- \rightarrow Fe^{2+}$ .771
$Fe \rightarrow Fe^{2+} + 2e$ .447

In chemical predictor, the oxidation for of $Fe^{2+} \rightarrow Fe^{3+} + e^-$ has an error in it?
it is reading $Fe^{2+} \rightarrow Fe^{2+} + e^-$

This is outright wrong.

$+2 \quad -2 \quad +2e^-$

$FeSO_4 + H_2O \rightarrow Fe^{+2} + SO_4^{-2} + H_2O$ Balanced
(This is not really redox, is it ionization?)

Now what does it take to go to Fe^{+3}

$Fe^{+2} + SO_4^{-2} + H_2O + H_2O_2 \rightarrow ?$

We have 3 tools for redox. Chem x
Chemical predictor
Chem tool Box (redox rxn only)

$Fe \rightarrow Fe^{+2} + 2e^-$
$2e^- + SO_4 \rightarrow SO_4^{2-}$

yes SO_4 is 2-
so it should be $FeSO_4$.

P108 Moore

We are learning that Integrators can lead to all kinds of reactions

from nothing essentially to forming electrolytes to forming precipitates to oxidizers & reducers.

We are talking here also about combining different ionic substances.

So I wonder how you know what happens?

Well the first question is whether or not something ionizes or not.

Well remember our electronegativity chart?!!

"Δ"

Δ	
0	covalent
.5–1.7	polar covalent
≥ 1.7	(none), ie ionic

e.g. NaCl 3.16 - .93 = 2.23

$FeSO_4$? polar covalent

Fe is 1.83
O is 3.44
S is 2.58

Δ = 0.86 with $\overline{X}$ = 3.26

3.26 - 1.83 = 1.43

polar covalent, might conduct some but is ~~not~~ expected to conduct ~~well~~ fairly strongly. You could test this

So we know that $Fe^{+2}SO_4$ does ionize. What we don't know yet is what happens when you add water.

Does it combine to form a weak electrolyte?

Does it combine to form a precipitate?

Does it cause a oxidation reduction reaction.

$FeSO_4 + H_2O \rightarrow Fe^{+2} + SO_4^{2-} + H_2O$

$Fe \rightarrow Fe^{2+} + 2e^-$ .447

$2H_2O + 2e^- \rightarrow H_2 + 2OH^-$ -.828

$E = -.381$

This reaction will not occur.

$SO_4^- + H_2O + 2e^- \rightarrow SO_3^{2-} + 2OH^-$ -.93

$Fe + 2H_2O + 2e^- + SO_4^- + H_2O + 2e^- \rightarrow Fe^{+3} + 2e^- + H_2 + 2OH^- + SO_3^{2-} + 2OH^-$

$= .447$
$-.828$
$-.93$
$-1.31V$ This reaction will never occur.

In contrast however if we look at

$Fe + H_2O_2$ this reaction will occur and lead to Fe^{+2}

also

(error in Chemical predictor) $Fe^{+2} + H_2O_2$ reaction will also occur and leads to Fe^{+3}

Now what about with water vs peroxide?

will occur + + 2.23V $Fe + H_2O_2 + 2H^+ + 2e^- \rightarrow Fe^{2+} + 2e + 2H_2O$

Well 1.01V occur $2(Fe^{2+}) + H_2O_2 + 2H^+ + 2e^- \rightarrow 2Fe^{3+} + e^- + 2H_2O$

So now we know it occurs in peroxide. What about water?

NO will not occur $Fe \rightarrow Fe^{2+} + 2e^-$

$2H_2O + 2e^- \rightarrow H_2 + 2OH^-$

No will not occur $Fe^{2+} \rightarrow Fe^{3+} + e^-$

$2H_2O + + 2e^- \rightarrow H_2 + 2OH^-$

This is counterintuitive but it says pure iron will not oxidize in water. This is amazing.

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So a great question

Will iron rust in pure water?
My analysis says no.

The web also says that sulfites (SO_3) will oxidize to sulfates (SO_4) in water.

Notice: sulfates are already oxidized to their final state.
Sulfates are oxidized sulfites!

The Chemical Predictor is very useful!

~~$SO_3^{2-} + 2OH^- + 2H_2O + 2e \rightarrow SO_4^- + H_2O + 2e + H_2 + 2OH^-$~~

~~$SO_3^{2-} \rightarrow SO_4^- + H_2$~~ Not true

$SO_3^{2-} + 2OH^- + 2H_2O + 2e \rightarrow SO_4^- + H_2O + 2e + H_2 + 2OH^-$

This should cancel to:
$SO_3^{2-} + H_2O \rightarrow SO_4^- + H_2$

Is this balanced?
4 O
2 H
1 S yes

So sulfite + water
gives sulfate + Hydrogen gas.

Ok, you are making progress.

Your new chemical will tell you how much Fe^{+2} vs Fe^{+3} is in hemoglobin.

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Now if it does not oxidize, also what does it do?

1. Dissociate?
2. Precipitate
3. Form an electrolyte?

What are the choices?

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Lets start identifying the chemical reactions involved.

Fe^{+2}

Fe^{+3}, H_2O_2

HbO_2

$HbO_2 \rightarrow Hb + O_2^-$

$Fe^{+2} \rightarrow Fe^{3+} + e^-$

$2(Fe^{2+}) + H_2O_2 + 2H^+ + 2e^- \rightarrow 2Fe^{3+} + e^- + 2H_2O$

~~Hb~~

So

$O_2 + e^- \rightarrow O_2^-$????

this electron gets added to molecular oxygen as an unpaired electron in its molecular orbital.

Does this lead to?

$2(Fe^{2+}) + H_2O_2 + 2H^+ + e^- \rightarrow 2Fe^{3+} + 2H_2O$

Our Space sh. goes 3.22E7 m/sec hour
It would take us 4.5 years to get there.

Astronomy: 300,000,000 m/sec

Mark asks some good astronomy questions

1. How many minutes for light to Saturn
2. How about stars? (visible magnitude)
3. how many stars in a galaxy?
4. How many galaxies known?

1. Saturn 900 million miles from sun
earth 93 million
Δ = 890 - 93 ≅ 800 E6 miles = $\frac{1.29E12\ m}{3E8\ m/sec}$
= 4291 sec = 71 min

2. Time to reach stars:
(2) Closest galaxy is 2.5 million light years - Andromeda
(1) Center of our galaxy is 26,000 light years away.
(3) Sh. M101 Black Night 20 million light years away
(4) Known universe: hundreds of millions of light years away.

3. How many stars in a galaxy?
Anywhere from a few million to several trillion stars in a galaxy.

4. More than 100 billion galaxies now known in the universe.

Thermochemistry Page 293

Predicting Reactions (on Arrow) (Masciotta) E2 Chemistry looks very useful. Chap 8, P193

Heat of formation looks to be critical information.

but Chemix has also a section on thermochemistry and they give an example on iron oxidation.

$4Fe(s) + 3O_2(g) \rightarrow 2Fe_2O_3(s) + 1647kJ$

There are in elemental form! $\Delta_f H = -1647kJ$

which means elemental oxygen iron in the +2 state (ferrous) + oxygen (in the -2 state) combine to form Ferric oxide.

Chemix is great. It has a very good table.

$\Delta_f H°$ is the quantity needed. -602.1 KJ/mol for MgO

$O_2(s)$ is $\emptyset$] but when you mix them
$Fe(s)$ is $\emptyset$] you have set a reaction.

$Fe^{+2}(aq)$ is -89.1

$Fe^{+3}(aq)$ is -48.5

$Fe_2O_3(s)$ is -823.5

$Fe(OH)_2(s)$ is -569.4

$Fe(OH)_3(s)$ is -823.5

Astronomy RMSS
Where is M13? www.thinkastronomy.com
(who is the speaker) Bill

1. bpoil disaster : gw tube
2. Steve Svenson - compiler
3. Craig Venter

Looking @ Thermochemistry
(Predictor in Chemistry)

$Fe^{+2}(aq)$
$H_2O_2(aq)$
$Fe^{+3}(aq)$

Lets back up on our oxidation reaction
$Fe^{+2} \rightarrow Fe^{+3} + e^-$

$Fe^{2+} \rightarrow Fe^{3+} + e^-$ $-.771V$
$H_2O_2 + 2H^+ + 2e^- \rightarrow H_2O$ $1.776V$
gw had an error

Net reaction is
$2(Fe^{2+}) + H_2O_2 + 2H^+ + 2e = 2(Fe^{3+}) + 2e + 2H_2O$

Notice the electrons cancel out

$$2(Fe^{2+}) + H_2O_2 + 2H^+ = 2(Fe^{3+}) + 2H_2O$$

Reaction will occur
$E_{cell} = +1.005V$

H does not matter what the SO_4^{2-} does.

Now the question I have is can the same reaction be predicted by thermochemistry (ie oxidation/reduction)

"Solutions of electrolytes are, in reality, solutions of the ions of the electrolyte. Therefore the chemical reactions of electrolytes are in fact the reactions of the free ions in solution."

Incredibly important statement

→ Thermochemistry is concerned with the formation of compounds. I do not know if it can be used to approach ions and redox reactions. Yes it can apply
See Mascetta p204 - Barrons!

Lets work out the oxidation reaction:

$4\overset{+3}{N}\overset{-3}{H_3} + 5\overset{-2}{O_2} \rightarrow 6\overset{+2}{H_2}\overset{-2}{O} + 4\overset{+2}{N}\overset{-2}{O}$
(g) (g) (g) (g)

How can something have $\Delta H = 0$?

You dont even need to understand the reaction to see if it occurs. You can just add up heats from Chemtex:

$(4(-45.9) + 5(0))$ ← minus this section. $[6(-241.8) + 4(90.2)]$ ← This section

-906 kJ

yes it will definitely occur.

ΔH (Products - Reactants)

to use thermochemistry

Reactn must be balanced

Now lets look @ $FeSO_4 + H_2O_2$ again

$2(Fe^{+2}_{(aq)}) + H_2O_{2\,(l)} + 2H^+_{(g)} \rightarrow 2(Fe^{+3}_{(aq)}) + 2H_2O_{(l)}$

ΔH(Products − Reactants)

$$\left[2(-48.5) + 2(-285.8)\right] - \left[2(-89.1) + (-187.8) + 2(0)\right]$$

≈ -302 kJ/rxn

says positively the reactn will occur.

Two separate methods now to say, it happens
Redox & thermochemistry

Your reactions must be balanced before yr proceed with these!!

So the question now is
$FeSO_4 + H_2O \rightarrow$? we know it ionizes

$\overset{+2}{Fe}\overset{-2}{SO_4} + H_2O \rightarrow$

First off, does it ionize?
(well essentially all salts ionize)
but in terms of electronegativity...

Fe 1.8 S↔O $\left(\frac{3.5 + 2.5}{2}\right) = 3$
S 2.5
O 3.5 F→SO $(1.8 - 3) = 1.2$

not exactly an accurate predictor

0 − .5 Covalent
.5 − 1.7 polar Covalent
> 1.7 Ionic

$FeSO_4 \rightarrow Fe^{+2} + SO_4^{2-} + H_2O$

Now we are learning that ionize to some degree also.
Water ionizes very weakly to

This is ionization / dissociation $2H_2O \rightleftarrows H_3O^+ + OH^-$ Ionization Constant = 1E-14
So it is very low.

Your question that has arisen is whether or not

$Fe^{+2} + H_2O \rightarrow Fe^{+3}$?

So what exactly is the reaction?

Need to balance $Fe^{+2} \rightarrow Fe^{3+} + e^-$ oxidation
$H_2O + 2e^- \rightarrow H_2 + 2OH^-$ reduction
This reaction does not occur, ??
notice the difference w/ $2H_2O \rightleftarrows H_3O + OH^-$

~~Fe+2~~ Chemical Predicts tells us that this reaction will not occur.

Combine 2 ???
$Fe^{+2} + H_2O + 2e^- \rightarrow Fe^{3+} + e^- + H_2 + 2OH^-$
It needs to be balanced before examining thermochemically.

The reaction does not occur. Therefore it can not be balanced.

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Guess what a reaction will take place in Oxygen, but not water. ~~free~~ Oxygen is a powerful oxidizer.

~~2(Fe^{2+})~~ No

It will happen with ozone! not free oxygen.

$2(Fe^{2+}) + O_3 + 2H^+ + 2e \rightarrow 2(Fe^{3+}) + 2e + O_2 + H_2O$

So again, it needs a powerful oxidizer to oxidize from Fe^{2+} to Fe^{3+}

Ozone, peroxide will cause it to oxidize

$FeSO_4 + O_2 \Rightarrow FeSO_3 + 4SO_3$

$FeSO_4 + H_2O \Rightarrow Fe(OH)_2 + SO_4 + H_2O$?

balances $FeSO_4 * 5H_2O + H_2O \Rightarrow Fe^{+2} + SO_4^{2-} + 6H_2O$

now that you have Fe^{+2}, what happens when you combine it with water?

$Fe^{2+} + H_2O \rightarrow$?

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You cannot find all redox reactions in ChemCalc predictor.

Trying to find oxidation of ammonia

$4NH_3(g) + 5O_2(g) \rightarrow 6H_2O(g) + 4NO(g)$

A Great Chem Lab (simulator) on
Ionic & Covalent Bonds.
Involves 6 Chemicals, 6 watch glasses,
6 bunsen burners, 12 test tubes, 6 in
water, 6 in ethanol, 6 beakers
6 Conductivity meters

		Chemicals are:	Melt	Soluble Ethanol	Soluble Water	Conductivity
$CaCl_2$	1	Calcium Chloride	NO	no	yes	143uS
$C_6H_8O_7$	2	Citric Acid	yes	yes	no	none
$C_{13}H_{10}O_3$	3	Phenol Salicylate	yes	yes	no	none
KI	4	Potassium Iodide	NO	no	yes	102uS
NaCl	5	Sodium Chloride	NO	no	yes	236uS
$C_{12}H_{22}O_{11}$	6	Sucrose	yes	yes	yes	none

Electronegativity	1.	1.0 - 3.2 = 2.2	$CaCl_2$	Ionic	143uS
	2				
	3				
	4	0.8 - 2.7 = 1.9	KI	Ionic	102uS
	5	0.9 - 3.2 = 2.3	NaCl	Ionic	236uS
	6				

0 - 0.5 Covalent
.5 - 1.7 Polar Covalent
$\geq$ 1.7 Ionic

Conductivity and/or electronegativity, seems to give the answer alone as to whether something is ionic or covalent. But I am sure there is more to the story.

. . .

Results, and these are important

Melting.

Notice some compounds do not melt easily.

Notice covalent compounds are soluble in ethanol, not soluble in water

Notice some compounds are soluble in water, only sugar as a covalent compound is soluble in water.

Notice ionic compounds are conductive and covalent compounds are not

These are important characteristics.

This is a great example of a lab that would have taken a lot of work to set up. The simulator told us all we needed to know.

Now what happens with hair - sulfur bonds?
Sulfur bonds must be very strong - why?

5 different ways of looking @ the same result.

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Notice our filaments are very hard to break down and they use iron.

Sound familiar?

1. Titanic chemistry?
2. Hair perm? Proteins w/ Sulfur?
3. Look for sulfur!

Move p169 sulfur elements burn w/ a blue flame

Hypo in photoshop is sodium thiosulfate $Na_2S_2O_3$

Beers law can be formulated as

Ratio of Concentrations = Ratio of Absorbances

This is an eminently more practical form to use & remember.

We now have a test for the sulfate ion.
$SO_4^{-2} + HCl + BaCl \rightarrow BaSO_4$ white precipitate

You are still correct w/ your barium test. Green flame can occur apparently for both copper and barium. But copper sulfate is soluble & your compound was not.

Page 303

An acceptor of electrons is an oxidizing agent.

like H_2O_2

The substance that it acts upon is oxidized, meaning that it has lost an electron.

Testing the sulfite-sulfate ion in blood:

When you test liquid iron for sulfate ion it passes immediately.

add $FeSO_4$ dilute (liquid iron) to H_2O
add dilute HCl
add 0.1 $BaCl_2$
immediate strong white precipitate formed

To blood,
the test fails completely
then indicates no sulfite ion in blood.

How about Fe^{+2} or Fe^{+3}? directly?

Blood Reaction:

I am getting a very interesting result.

1. Blood diluted in water. (This is the "apt" test
2. Add NaOH 1-2 drops
   turns the blood a light green color
   (indicative of Fe^{+2} ??)
3. Add H_2O_2 in attempt to produce Fe^{+3}
4. Instead I am getting some kind of
   filament produced?
   Solution also turns clear

1,10 phenanthroline has a molecular wgt of
180.209 gms

Assume we would like to use .2gm in 60ml

(.2) $\frac{180.209 \text{ gms}}{1 \text{ Mole}} = \frac{X}{60 \text{ ml}}$ $X =$

$\frac{.2 \text{ gms}}{60 \text{ ml}} = \frac{X}{1000 \text{ ml}}$ $X = 3.33\overline{3} \text{ gms}$

and $\frac{3.33 \text{ gms}}{180.209 \text{ gms}} = .018 \text{ M}$

Pase 305

Looks like Standard reagent is Ø.1 % wgt/volume

60ml = 60gms

Ø.1 % = .001(60) = .06 gms

Solvent is dilute acid hydrochloric acid

It is also soluble in water to Ø.3 %
and Ø.3 % is a solid solution

.003(60gms) = Ø.18 gms This is fine use it.

Sodium Thiocyanate NaSCN
Molar Mass = 81.07 gms/mole
highly soluble 139 gms/100 ml.

so a
.1M solution is .1(81.07) gms/1000ml = 8.11 gms/1000ml

for us @ 60 ml

$\frac{8.11\text{gms}}{1000\text{ml}} = \frac{x}{60\text{ml}}$ $x = .486\text{gms} = .49\text{gms}$

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Blood tests for Fe^{+2} or Fe^{3+}:

Both tests fail. This is fine.

First test is for free ions of Fe^{2+} in blood using 1,10 Phenanthroline. This test fails.

This means there are no free ions of Fe^{2+} in the blood.

Now we test for Fe^{3+} using sodium thiocyanate. The test fails. This means there are no free ions of Fe^{3} in the blood.

This is fine. The iron is bound in the blood. It should not be free.

But now we try the culture.

Now the Culture test fails also for Fe^{2+} & Fe^{3+} using 1,10 & NaSCN

This simply means there are no free ferrous or ferric ions in either the culture or the blood.

This does not mean there is no iron, only that in the blood & the Culture the ions are not free.

But your thesis is that the spectrum of the culture essentially matches that of Fe^{+3} in solution.

So How Can this be??

You now have 3 ferric salts. Test them.

We do have a problem. Culture peaks in the yellow region.

Blue Yellow Green Red
Culture absorbs in the blue portion of the spectrum.

Culture shifts absorption to the right (This means a shift in transmittance to the left, or towards the blue.

Fe^{3+} Reasoning

Synopsis.

We have a little problem, a kink in the reasoning process.

We cannot say that the spectrum of the Culture "matches" the spectrum of the Fe^{3+} ion.

This actually is as it should be because we know already by is unique.

What we can say is that it has similar general properties to the spectrum of ferric salts

eg.
ferric ammonium sulfate
ferric chloride
and ferric nitrate

w/ a sharp drop off @ 397 nm and a strong general decline in absorpance as we head toward longer wavelengths.

~~We can also~~
~~the Greeks say that a linear continuation~~
We also based a model upon the use of ferric ammonium sulfate & reverse hemoglobin.

Question, what happens w/ higher concentrations of the ferric sulfate salts?

ferric ammonium sulfate is looking different than the other salts.

Now ferric ammonium sulfate needs to be filtered. Why? I am not sure but it does and it makes a difference. (impurities suggested?)

The is now getting very interesting.

Ferric Ammonium sulfate $FeNH_4(SO_4)_2$ IS indeed surprisingly close. What does that mean NH_4? SO_4^{2-}? Are there also factors.

If it is concentrated the second peak appears. It looks like a stronger solution produces a stronger peak & shifted to the right.
Moderate concentration 2nd peak @ 426
High concentration " @ 440.
surprisingly close

Question: from our math model why do we have a peak in our model @ 420 vs 440???

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Topics:
1. Evaluate strongly colored solutions of ferric salts
2. Examine NH_4 & SO_4 influences
3. Titanic Chemistry —

We have made an important adjustment in the model.
We have eliminated the primary H_2O_2 reference peak @ 414 nm since it does not fit both the $FeNH_4(SO_4)_2$ and the meas. avg blood spectrum.
It is distorting the model.

You now have new unknowns of

$$\Delta = \begin{bmatrix} .464 \\ .906 \end{bmatrix} \quad \text{and} \quad Q_{xx} = \begin{bmatrix} .102 \\ .125 \end{bmatrix}$$

and new $\Sigma v^2 = 1.545$

$n = 13$

$\sigma = .343$ $\quad \sigma^2 = .118$

so $\Sigma_{xx} = .118 \begin{bmatrix} .102 \\ .125 \end{bmatrix} = \begin{bmatrix} .021 \\ .015 \end{bmatrix}$

so

$$\sigma_{\Delta_1} = .145$$
$$\sigma_{\Delta_2} = .122$$

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$B\Delta = f - v = f + (-v)$

Our new model values are: Model! Old Model

λ	f	v	-v	f + (-v)	
340	0	0	0	0	0
366	.10	-.60	.60	.70	.64
397	1.28	.04	-.04	1.24	1.13
401	.66	-.41	.41	1.07	1.06
442	1.49	.29	-.29	1.20	1.09
452	.95	.08	-.08	.87	.79
471	.65	.11	-.11	.54	.50
509	.41	.08	-.08	.39	.35
542	1.42	.34	-.34	1.08	1.02
560	.90	-.08	.08	.98	.93
577	1.42	.13	-.13	1.29	1.20
600	.06	-.29	.29	.35	.33
645	-.01	.24	-.24	-.25	-.23
700	0	-.008	.008	.008	-.007

Still some quirks. Ref HbO_2 $FeNH_4SO_4$

Practical in model: Purple = Black + Red

You need more data points.

You might be able to skip the mask model. The mask model does not exactly catch everything.

What we see is that $FeNH_4(SO_4)_2$ captures the measured bilood very well whatever it has influence (magnitude).

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Ok, the lesson now is that $Fe(NH_4)(SO_4)_2$ amazingly captures the majority of the spectrum where it has absorbance impact (ie 340 - 500 nm).

We know this is likely due to the Fe 3+ ion because of 3 ferric salts studied

1. ferric ammonium sulfate
2. ferric chloride
3. ferric nitrate

and all have a peak @ 391.

We also know that Fe^{+3} is likely involved because of the culture turning a dark, brown color due to metabolism using $FeO_2 + H_2O_2$ which forms Fe^{+3}. This means that it is likely consuming more in the Fe^{+3} state.

If there is more in the Fe^{+2} this leads to an expectation of methemoglobinemia. Initial data indicates there may be a validity.

Scanning may be an best tool.

Question now coming up is what is the possible influence of NH_4 & SO_4 ions?

There is both ferrous and ferric ammonium sulfate so be careful!

Ferrous: $Fe(NH_4)_2(SO_4)_2 \cdot 6H_2O$

ferric: $Fe(NH_4)(SO_4)_2 \cdot 12H_2O$

$(+3)\ (+1)\ (2(-2) = -4)$

What is oxidation state of NH_4? (+1)

SO_4 (2−)

Fe^{+2} & Fe^{+3}

You must look @ the other salts in above Concentration form.

Let's look @ conductivity: Ω

$FeNH_4(SO_4)_2$	70 Ω	most conductive
$FeCl_2$	89 Ω	high most conductive
$Fe(NO_3)_3$	220 Ω	least conductive
NaCl	19 Ω	the most!

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$+3 +1 -2(2) = -4$

Now we know that $FeNH_4(SO_4)_2$ dissassociates. do it ionyzls.

so we should have

$FeNH_4(SO_4)_2 + H_2O \rightarrow Fe^{+3} + NH_4^{+1} + 2SO_4^{2-}$

You could test for the ion.

~~Still~~ need to test other ferric salts as it relates to concentration

Fe^{+3} ~~tests~~ absolutely positive w/ sodium thiocyanate very useful test!

SO_4^{2-} ion? Dilute HCl $BaCl_2$ absolutely tests positive

very good tests.

Then means we must have the ammonium ion.

This is critical. [The Fe^{+3} ion is yellow in color.
~~Fe+2~~ ion is colorless This is false!
I don't know ~~that~~ by this.
Fe^{+2} is green! Remember liquid iron.

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$NH_3 + H_2O \rightarrow NH_4 + OH$

Household solution is a solution of
NH_3 in water.
and we know that creates the ammonium ion
which is colorless as in the salt we started

Household ammonia is also called
"ammonium hydroxide"
Now you know why

The last one is the SO_4^{2-} ion.
Does it have a color?

Problem: If the spectrum of $FeNH_4(SO_4)^{2-}$ (+3 +1)
matches that of blood
??? Then why does the blood test fail
for the presence of Fe^{+2} ion?

Actually blood + NaOH did turn to
perfectly clear

We can prove that SO_4^{2-} is colorless by
dissolving $MgSO_4$
You did as colorless.

Magellan: A Developing Thesis

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Therefore we know the color of the $FeNH_4(SO_4)_2$ spectrum is due essentially exclusively from the Fe^{+3} ion.

This leaves 2 questions:

1. What happens w/ more concentrated $FeCl_3$ and $Fe(NO_3)_3$?

Do we get the second peak?

2. Does blood contain Fe^{+2} or Fe^{+3} ion?
How can the spectrum of the ion $Fe^{+3}NH_4^{+1}(SO_4)_2^{2(-2)}$ match that of measured blood.

A Color question.
Change in the blood in more extreme cases seems to push absorbance to the right, or longer wavelengths.

ie seems to push it (absorbance) from violet to blue - to blue green

If blood is turning purple

It means you are adding a component of absorbance in the yellow portion of the spectrum (approx 550 nm).

Notice in our comparison of measured blood, the peaks @ 550 have been accentuated.

In addition to magnitude from 350 to 450 has been diminished. (This is absorbance wavelength). This corresponds to seeing less of yellow.

Therefore from the magnitude argument, the spectrum would suggest you would expect to see more violet (purple) in the blood ~~and less~~ @ 550 nm

and less violet absorbed @ 400 means less yellow color observed.

The other argument
Comes from methemoglobinemia.

Proving the Fe^{+3} case.

We are now reconfirming that it is indeed the Fe^{3+} ion that is responsible for the peak structure that is being observed.

We have prepared a strong $FeCl_3$ solution and the peaks are as strong as ever

397 $\Delta = 50$ nm
447 $\Delta = 57$ nm
498

A very strong positive result here. Yellow iron salt did not do this.

Proven again with Ferric Nitrate

Strong Peaks @ 397 & 448

The case is made that the Fe^{+3} ion is responsible for our peak structure.

The APT test is a test for fetal vs adult blood.

Blood + NaOH = denatured for adult (turns yellow-brownish)

Blood + NaOH = pinkish for fetal hemoglobin

So, it is "denaturing" the protein of the blood. What does this actually mean?

Denaturing means the proteins change shape

Let's try the salts of the spectroscope.

Estimate of Fe^{+2} concentration

Fe^{2+} by wgt = 3.25%

1 mole = $FeSO_4$ =

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Chemicals needed

~~Kmno4~~ Potassium Permanganate - God, I bought it!
Sulfuric Acid
Nitric Acid

$KMnO_4$ - I have 30 gms

Molar mass = 158.027 gms/mole
Assume we have a 60 ml bottle
Solubility is 6.38 gms/100 ml — OK

Standard solution = .0484 M ?

$.0484\ (158.024) = \frac{7.648\ gms}{1000\ ml}$

So $\frac{7.648\ gms}{1000\ ml} = \frac{X}{60\ ml}$ $X = .459\ gms$

$= .46\ gms$

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$V + B\Delta = f$

$B\Delta = f - V = f + (-V)$

Our new model values are: Model: Old Model

λ	f	V	-V	f + (-v)	
340	0	0	0	0	0
366	.10	-.60	.60	.70	.64
397	1.28	.04	-.04	1.29	1.13
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Still some quirks. Ref HbO_2 $FeNH_4SO_4$

Practical in model: Purple = Black + Red

You need more data points.

You might be able to skip the mask model. The mask model does not exactly catch everything.

What we see is that $FeNH_4(SO_4)_2$ captures the measured blood very well whatever it has influence (magnitude).

Lets try and titrate the iron (liquid iron) lets divide.

Take 2ml of Liquid iron

Use 2ml of liquid iron.
add 8ml of water
$\Sigma = 10ml$

Now add $KMnO_4$ by drop

You cannot seem to get a sudden color change.
only a gradual change in the color.

Not sure why?

Time for Ligands & Cordination Chemistry.
p. 1075 - Brown

We notice that liquid iron passes the test for Fe^{2+} existence, and yet fails the titration attempt to determine concentration based upon presumption of Fe^{2+} existence. Why?

Can "metal complexes" be determined by ion tests? Why does blood not test positive for Fe^{2+} & Fe^{3+} - is it because it is in a complex? Same thing for liquid iron as it is chelated (chlorosis)?

Good questions - let me go.

Complex metal complex (neutral)
Complex ion (charged)

Now we understand metal complex
Notation: [metal complex] total charge

so what is
~~Fe NH4~~ etc FeN_4??

Fe^{+2}
N is -3

So FeN_4 is coordinate covalent w/ 2 Nitrogen & 2 nitrogen donors so

$Fe^{+2}N_2^{-6} = -4$

And the electron donor from the other nitrogens are 2 apiece, which leads to 4 so the net balances out to zero.

~~so the six~~
ligands bind to the

We understand are have the proposal that Fe^{+2} are changed "somehow" to Fe^{+3} w/in heme.

We have no idea what "somehow" means here. Other than an oxidation takes place eg w/ H_2O_2 apparently ? . . .

Now let's go back & try to learn about the structure independent of this. We had made some progress.

Ok, some progress

Iron is oxidized here

We have just answered a lot of questions.

Acetic acid oxidizes blood.
This means it changes from Fe^{+2} to Fe^{+3}.
It is still bound, it is not an ion.
Fe^{+3} ion test fails, which you now know it should.
It turns brown as it should.
No salt is needed, this forms a chloride

Add plenty of salt and it forms Iron Chloride. Insoluble in water!
Makes a dark brown precipitate.
Dark brown precipitate.

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Hemin itself is a chloride of heme.
It is a chelate of heme.
It is not oxidized heme by itself.

I wonder how sensitive the test is?

2 drops of highly diluted blood are barely detectable if you look very closely.

So I would regard the test as fairly quite sensitive.

Hematin is $C_{34}H_{33}FeN_4O_5$

An important statement from Justice Dept Document.

* Methemoglobin does not bind oxygen but will bind a number of other ligands such as

hydroxide (OH^-)

cyanide CN^-

azide N_3^-

nitrite (NO_2^-)

Pulse 325

Fuel Raising an apple one jar head is about 1 joule

Raising 4540 apples
= 50 apples per day for 3 months.

So 4540 Joules
= 4540 watts in one second
= 15 watt bulb for 5 minutes

Fe Ionization Potential

1st 759.3 KJ/mol
2nd 1561.1
3rd 2957 KJ/mol

of Fe^{+2} 4 per hemoglobin molecule how often

humans have roughly 2.5E13 red blood cells.

~~roughly a 1/3 of total volume is hemoglobin~~
~~≈ 210 E6 molecules of~~

≈ 280 E6 molecules of hemoglobin in each red blood cell

So 280E6 (2.5E13 cells) = 7E21 molecules of hemoglobin

x4 heme per ea hemoglobin molecule = 2.8E22 Fe^{+2} atoms in the human body.

$(2.8E22\ Fe^{+2}\text{'s})$ (no damage) (.07) (~~2957-1561~~ kJ

= 1.96 E21 Fe^{+2}'s are damaged

$5^{00} =$
3.24 KJ
= 3240 KJ

No moles = $\frac{1.96E21}{6.02E23}$ = .00326 moles??

.00326 (2957 - 1561 KJ) = 4.54 KJoules

= 4540 Joules

one watt is one joule per second

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To ionize something is to impart a charge.

Mass spectrometry requires a gas.

Culture fails Fe^{3+} and Fe^{2+} test.

Just like blood does
The says no ionic form

suggests a metal complex form.

How do we test for metal complexes?

Will the iron tests determine metal complexes?

How to form metal complexes and test them? Remember blood fails Fe^{3+} and Fe^{2+} tests also.

$AgNO_3 + NH_4 = ?$ Can we get $AgNH_3$?
No color reaction

Conductivity:

1 drop of culture in 10 ml of H_2O = 5 µS.

Now NaOH 2 drops in 10 ml H_2O is 130 µS!

So How did we make our culture?

Ok, we have found the Concentration of our culture

We have Concentration = 14.61 mg/ml.

Now to make this we put

$$\frac{20 \text{ drops NaOH}}{105 \text{ ml of water} - (20 \times .06 \text{ ml})} = \frac{20 \text{ drops NaOH}}{103.8 \text{ ml } H_2O}$$

$$\text{So } \frac{20 \text{ drops NaOH}}{103.8 \text{ ml } H_2O} = \frac{1 \text{ drop NaOH}}{X} \quad X = 5.19 \text{ ml } H_2O$$

but we just put 2 drops NaOH in 10 ml H_2O which is about exactly the same ratio and get NaOH @ the Concentration & Conductive @ 130 µS. But we only get 5 µS. Why?

Because we are putting 1 drop of culture in 10 ml of H_2O, so we are highly diluting it.

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So now the logical question is what is the conductivity of the concentrated stock solution by itself?

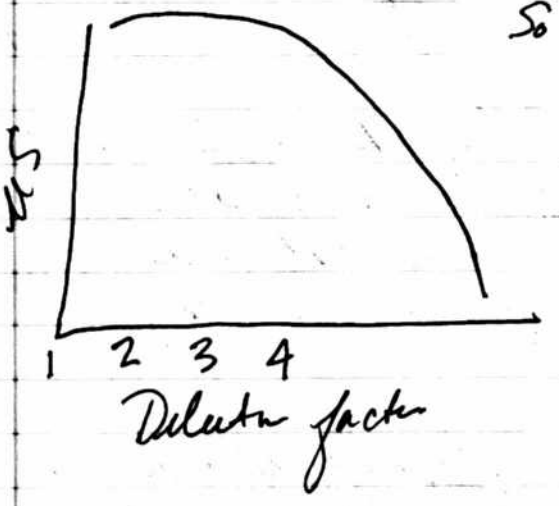
10 ml — Weigel 1540 uS. vs 1387 uS. This is close.

20 ml — Dilute by factor of 2. It reads 1 which mean it is too high?

25 ml — by factor of 2.5 reads 1660

30 ml — #3 1400

40 ml — 4 950



So the conductivity may have this form. It is not linear.

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Now the question is Can it somehow precipitate out the Na^+OH^- ions?

We think our culture we have
$(Fe3+ \rightarrow ligand) + Na + OH^-$

KNO_3, $CuSO_4$?

$CuSO_4$ is Cassy c reaction w/ the culture.
We have a precipitate

$Fe(OH_2)(s)$ is a dark green precipitate

$Fe(OH)_3$ + is a brown precipitate

It is looking like the culture is producing ferric hydroxide, not ferrous.

Prove it.

What we are learning here is that the culture does not react to Fe^{2+} or Fe^{3+} tests. That means the Fe does does not exist in ionic form. That is the same as blood. Repeat that test.

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However the cultures react w/ $CuSO_4$ to form a dark precipitate.

$CuSO_4 + Fe^{+2}SO_4 \rightarrow ?$ X

$CuSO_4 + Fe^{+3}SO_4 \rightarrow ?$ X

$CuSO_4 + Fe^{+2}X + H_2O \xrightarrow{?}$

$CuSO_4 + Fe^{+3}X + H_2O \xrightarrow{?}$

how about Ferric Chloride
Ferric Nitrate
ferrous

Can salt besides ferrous Sulfate?

We need a test for ferrous vs ferric hydroxide

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Now, Liquid Iron + $CuSO_4$?
No reaction occurring

$FeCl + CuSO_4 \rightarrow$?
also not reacting - why?

So why are setting a reaction with the culture?

$FeCl + NaOH + CuSO_4$? yes, positive reactions

Liquid Iron + $NaOH + CuSO_4$?

There is a reaction but nothing as dramatic as what you have in the culture

Baking Soda gives a reaction of $Fe^{+2}(OH)_2$

We now will have a complication.
Baking Soda turns the iron form back to the original form Ferrous or Ferric.
ie ferrous - turns back green
ferric - turns back orange

The problem: Our culture turned back green! We would indicate it is Fe^{+2} which is not what we thought.

Question: Is it reduced from the $CuSO_4$??

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We have an ~~extremely~~ statistic.

~~$CuSO_4$ + Culture = no reaction.~~

~~$CuSO_4$ + Culture + Lye~~

Liquid $Iron^{+2}$ + $CuSO_4$ no reaction

$FeCl^{+3}$ + $CuSO_4$ no reaction

Liquid $Iron^{2+}$ + $CuSO_4$ + Lye reaction

$Fe^{3+}Cl$ + $CuSO_4$ + Lye reaction

What reaction?

Culture + $CuSO_4$ may ppt precipitation reaction. believed to be Fe^{3+} OH because of brown color vs green

BUT when baking soda added, it returns green which indicates Fe^{3+}.

But! The second test the precipitate stays brown.

So it remains unclear.

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The color of the culture is brown, not green.

$CuSO_4$ added to the culture definitely caused a dark brown precipitate. This would indicate Fe^{3+} OH^-. looks like a fairly positive test to me.

Not to else is needed.

Fe ligand + Na + OH + $CuSO_4$ → ???

I would like to have another ferrous sulfate.

Test for Cu^{+2} ions?

In the ion form (we dont have this)

$Fe(s) + CuSO_4 \rightarrow Cu + FeSO_4$

Iron is more active than copper.

Ferric hydroxide is $Fe(OH)_3$

$FeCl_3 + 3NaOH \rightarrow Fe(OH)_3 + 3NaCl$

Water when bound to Fe^{3+} is highly acidic (wikipedia on rust)

Citrate solubilizes ferric ion at neutral ph

Sodium Citrate turns the Clear solution green and dissolves the precipitate!

Sodium Citrate is $Na_3C_6H_5O_7$

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Baking Soda + Citric Acid:

$3NaHCO_3 + C_6H_8O_7 \rightarrow C_6H_5Na_3O_7 + 3CO_2 + 3H_2O$

sodium bicarbonate — Citric acid — sodium citrate — Carbon dioxide — water

not directly relevant Ferric hydroxide + Citric Acid = ferric citrate

???? $C_6H_5Na_3O_7 + Fe(OH)_3 \rightarrow ? Fe^{+2} ? +$

Charge is −3 Citrate ion should be $C_3H_5O(COO)_3$

OK, ya may have something. Sodium Citrate appears to not dissolve ferrous hydroxide but it does dissolve ferric hydroxide,

Then might do it.

Citrate Ion appears to be $C_6H_8O_7$

$C_6H_8O_7 + Fe(OH)_3 \rightarrow ???$

Ferric Citrate

$C_6H_8O_7$ is citric acid.
Molecular Mass is 192.125 gms/mol

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Ok, we are learning
ferric hydroxide dissolves in
citric acid.

1. First take Cultan solution
1. Coltric + $CuSO_4$ → precipitate
released to be Ferric dehydroxide -

Molecular wgt of citric acid ($C_6H_8O_7$) is 192.125 gms/mol
We have a 30 ml bottle

$$\frac{192.125 \text{ gms}}{1000 \text{ ml}} = \frac{X}{30 \text{ ml}} \qquad X = 5.764 \text{ gms}$$
for a 1 M solution

Solubility is $\frac{60 \text{ gms}}{100 \text{ ml}}$ so we can make a 1 m solution

and

Precipitated $Fe(OH)_3$
Citric acid
$Fe(OH)_3 + C_6H_8O_7 \rightarrow$ dissolves!

$Fe(OH)_2 + C_6H_8O_7 \rightarrow$ also dissolves
but it goes back to
green Fe^{2+}

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We have proven that the culture contains Fe^{3+}, apparently in a complex form (ligand).

(1) Then done by precipitating $Fe(OH)_3$ w/ $CuSO_4$. (No match $Fe(OH)_3$.

(2) Dissolving in citric acid. Now goes into ionic form. Fe^{3+}

NO!!!

No it does not!!!!
It fails the Fe^{3+} test ionic

Why? Why? Why? Why?

It is necessary it is the original form but it is not ionic.

So what exactly is the reaction that is taking place here? It appears to be $Fe(OH)_3$ but could it be

$Fe-X-(OH)_3$? Something combined with Fe^{3+}

There is a conundrum here.
It does not seem to be ionic

Something about this is a mystery.
It is not ionic.

(1) Try the process w/ a known ferric salt.
$FeCl_3$

(2) Now precipitate out w/ $CoSO_4$

* Big lesson: It does not precipitate until we add NaOH. Why is this...???

(3) Carry on w/ dissolve in Citric acid

(4) And it also fails the Fe^{3+} ion test.

So this is good. Something is going on with the lye and the Fe^{3+} ion.
Notice w/ Fe^{2+} (liquid mixture going forward & reverse it certainly did not pass the Fe^{2+} ion test).

~~Ok, big problem.~~

Ok, we are getting a reaction from $FeCl_3$ + NaOH alone.
What is this reaction
It alone forms the precipitate $Fe(OH)_3$
nothing else is needed.
Citric ~~acid~~ acid dissolves.
Fe^{3+} test fails.
so citric ~~acid~~ acid does something to prevent the Fe^{3+} test from succeeding.

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So we have now learned that Citric acid, dissolves both ferrous & ferric hydroxide.

But something happens that does not allow Fe^{2+} or Fe^{3+} to react, as can't test for iron again, and also that Fe^{3+} test fails.

It does not mean it is not ferrous or ferric hydroxide, but only means they are not necessarily in ionic form after dissolving in citric acid.

Ferric hydroxide + Citric Acid = ~~Ferric Ammonium Citrate~~ Something Ferric Citrate

Something + Ammonium Hydroxide = Ferric Ammonium Citrate

Ferric Citrate = $Fe(C_6H_5O_7) \cdot nH_2O$
pale brown in color but have a match.

Ja figured out the reaction in Chem X

Figured in Chem X

$$Fe(OH)_3 + C_6H_8O_7 \rightleftharpoons Fe(C_6H_5O_7) + 3H_2O$$

Ferric hydroxide — Citric Acid — Ferric Citrate — water

So it is not an ion!!! But it is pale brown

The organism reaction is shown and proven!!!

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Ferrous Citrate is $FeC_6H_6O_7$

So the reaction is

$$Fe(OH)_2 + C_6H_8O_7 \rightarrow FeC_6H_6O_7 + 2H_2O$$

ferrous hydroxide — Citric acid — ferrous citrate — water

Color of ferrous citrate is: (gray green powder) green mottled brown
ferric citrate is: pale brown

So you have it.

Now the question: how does Culture + $CuSO_4$ → produce $Fe(OH)_3$??
(has NaOH)

$$Fe^{3+} + NaOH + CuSO_4 \rightarrow Fe(OH)_3 + H_2O$$

$$CuSO_4 + 2NaOH \rightarrow Cu(OH)_2 + Na_2SO_4$$ (+ Na + OH?)

I have the reaction from Chemix!!

$$2Fe^{3+} + 6NaOH + 3CuSO_4 + H_2O \rightarrow 2Fe(OH)_3 + 3Cu^{2+} + 3Na_2SO_4 + H_2O$$

Shown / proven

(visible) — tested

This is our reaction of the culture

Cu++ test: household ammonia

This reaction confirmed with NH_4OH

The Chemistry to verify Fe^{3+}

and then !!!

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Pale Brown Power Shown!!

$Fe(OH)_3$ + $C_6H_8O_7$ → $FeC_6H_5O_7$ + $3H_2O$

Ferric Hydroxide + Citric Acid → Ferric Citrate + water

You have proven the chemistry of the precursor is composed of significant Fe^{3+} in a metal complex not an ionic form

Now the final colors

This is the place to end the book.

Case will be proven by

1. Observation of Culture growth
2. Chemical analysis
3. Spectral analysis
4. Blood sampling color observation

Now the question of what it can bind with?

OH^- hydroxide

CN^- Cyanide a respiratory inhibitor

Azide N_3^- a respiration ~~cobalt~~ inhibitor

Nitrite NO_2^-

We know Fe^{2+} is bound w/ the FN_4 so it would be a simple matter to charge to $Fe^{3+}N_3^-$

FeN_3 What is the compound?

or would it be Fe_3N_3 ???

What is an azide?

The Azide anion is $\bar{N}=\overset{+}{N}=\bar{N}$

the azide functional group is $R-\overset{}{N}=\overset{+}{N}=\overset{-}{N}$

Azide can be bound to Hemoglobin.

End