DPD Colorimetric Testing for Chlorine

Using vacuum ampuls to test for total residual chlorine

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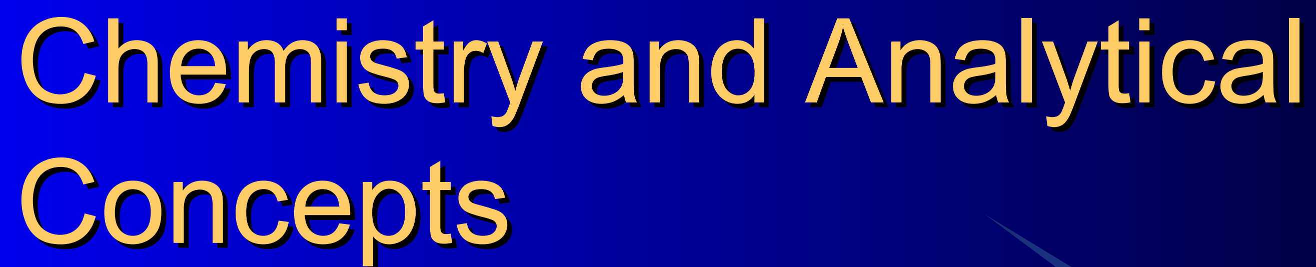
Overview

DPD Colorimetric Testing for Chlorine

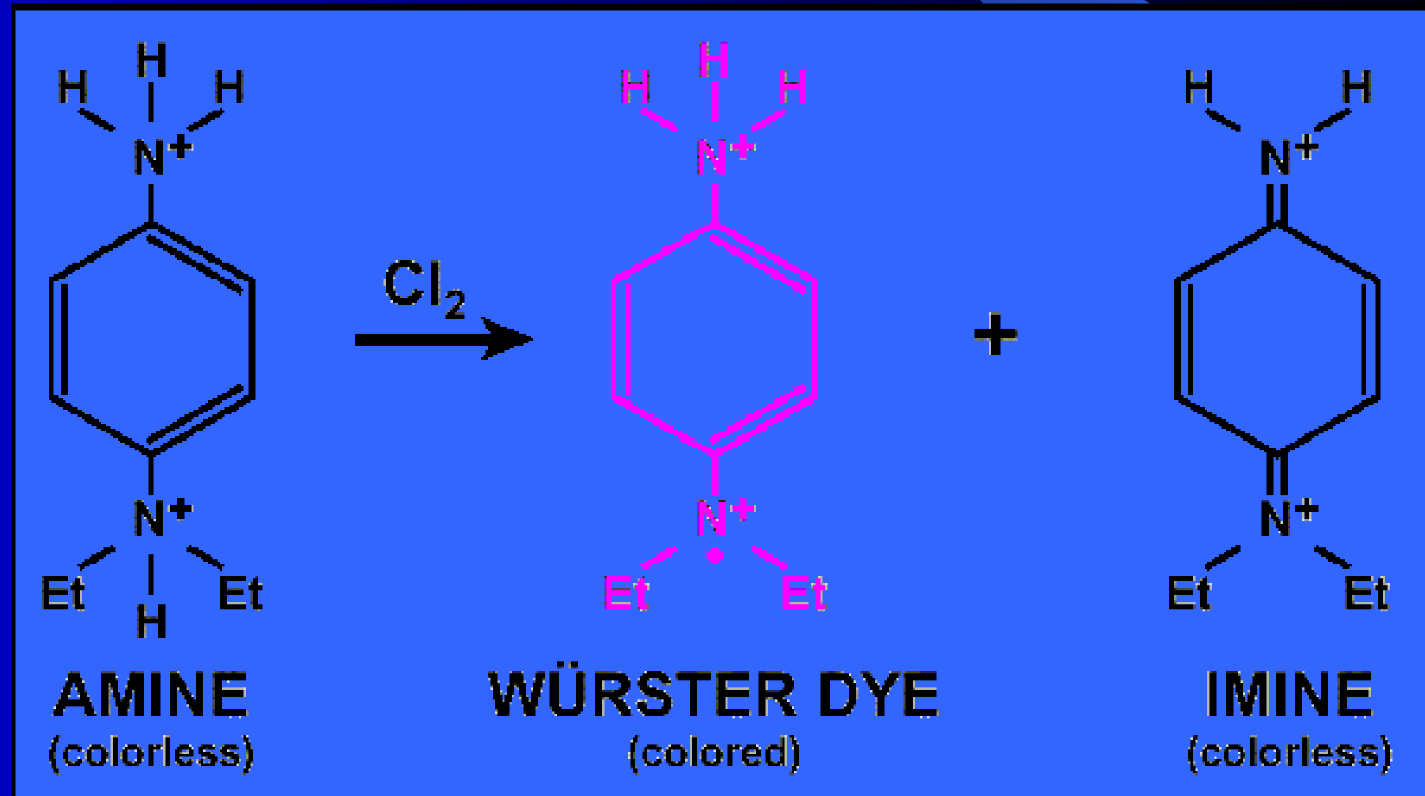
Using vacuum ampuls to test for total residual chlorine

- Chemistry and analytical concepts
- Methodology
- Procedure
- Summary


Chemistry and Analytical Concepts

What is DPD?

- DPD is N,N-diethyl-p-phenylenediamine
  - The amine DPD reagent reacts with chlorine stoichiometrically to from a Würster dye and an imine.
  - We see the magenta Würster dye.



What's a Vacuum Ampul?

- A vacuum ampul is a vacuum sealed spectrophotometric cell.
- The ampul is snapped open and a fixed amount fluid is drawn into the ampul.
- There are often reagents in the ampul (like DPD).

Benefits

- Reagents in vacuum ampuls are not prone to contamination due analyst handling.
- Less transferring and measuring of solutions. Solutions are made and read in the same ampul.

Technical Considerations

- Do not work in direct sunlight.
  - Chlorine (among other halides) is affected by intense light, leading to errors.
  - If working outdoors (on-site), be sure to work in the shade.

Technical Considerations

- Dilute precisely and accurately.
  - Solutions less than 0.1mg/l in concentration are especially vulnerable to error.
  - At low concentrations, errors as little as 0.05% can change absorbance measurements by over 15%.
  - Dirty glassware will significantly effect low-concentration solutions.

Class A automated pipets

Technical Considerations

- Use good pipet technique.
  - Ideally, use a automated class A pipet.
  - Use a new pipet tip for each solution.
  - Never pipet from the stock solution, this can contaminate the whole lot. Pour the solution into a smaller beaker first.
  - If using a pipet bulb, be sure not to draw solution into the bulb itself.

Technical Considerations

- Use consistent technique.
  - Dilutions
  - Timing
  - Readings
- Consistency will minimize errors and increase the potential to identify and correct errors.

Methodology

Approved and Acceptable Methods

NR 219	**NR 809**
EPA 330.5 *or* Std. Mthds. 4500-Cl G	Std. Mthds. 4500-Cl G *or* EPA approved methods

- NR 219 lists Standard Methods 4500-Cl G and EPA method 330.5 as the ONLY acceptable methods for **DPD** testing of total residual chlorine.
- NR 809 lists Std. Methods 4500-Cl G or any EPA approved method. Thus any approved method can be used under NR 809.
- These methods are very technically demanding and time consuming. There are many commercial methods available that simplify the procedure.

Approved and Accepted Methods

If a commercial method is accepted by the USEPA as equivalent to one (usually both) of the methods listed in NR219, than it can be used for reporting purposes to the WDNR for NR 219 (in addition to NR 809).

NR 219

EPA
330.5
OR
Std. Mthds.
4500-Cl G

NR 219.05

Chlorine--Total residual 8167
Chlorine--Total residual 8168
Chlorine--Total residual 10014

Commercial Test

Letter from USEPA indicating acceptance of commercial test method

If you are going to use a commercial test, their must be documentation that the test method is approved.

Approved and Accepted Methods

The following is a list of some commercial DPD methods that are USEPA accepted. This list is by neither exclusive nor complete.

- Hach Method 8021
- Hach Method 8167
- Hach Method 8370
- Hach Method 8371
- Hach Method 10014

NOTE: Although all of these methods use DPD, they do not necessarily use vacuum ampuls.

When using a commercial test, it is necessary to cite the SPECIFIC method (e.g. method number) you are using. A test category is not acceptable.

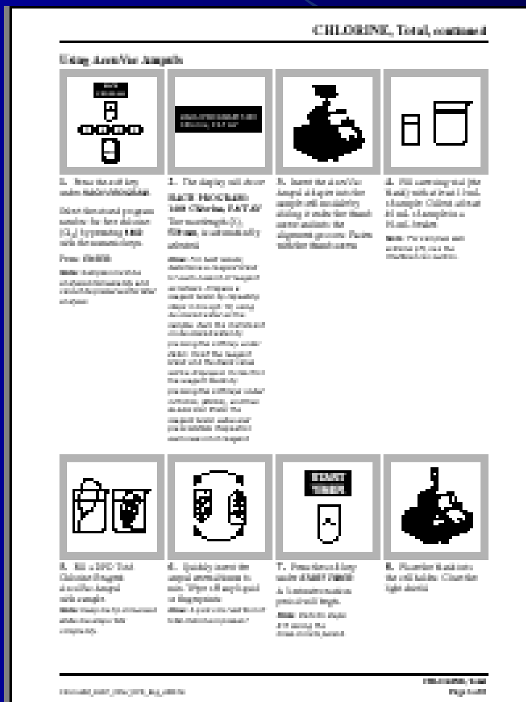
Approved and Acceptable Methods - A Note of Caution

- The commercial methods often gloss over the very important aspects of traceability, calibration, spikes, duplicates, and other QA and QC measures.
- Using a commercial method does not exempt you from the QA and QC established in the original EPA/Std. Methods:

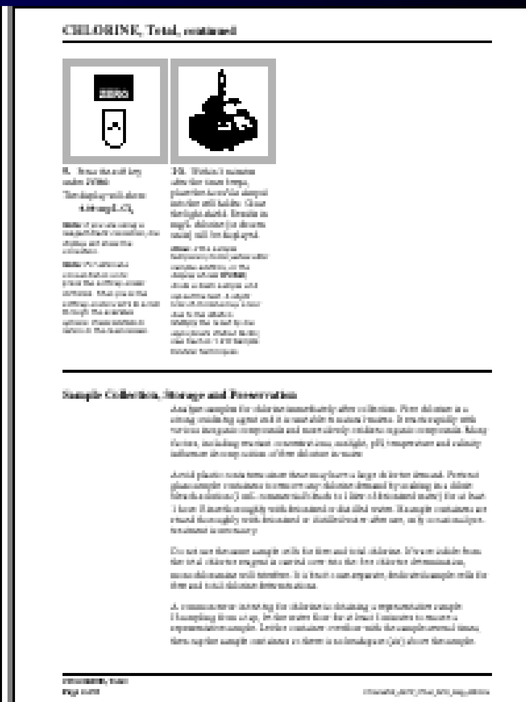
Commercial Method + QA/QC = Acceptable Testing

Commercial Methods - Thoroughness

- The exclusive use of generic instructions are not acceptable.
  - No true calibration
  - No QC
  - No spikes/dupes
- These instructions are useful for quick checks and summary only


CHLORINE, Total, continued

Using AccuVac Ampuls


CHLORINE, Total, continued

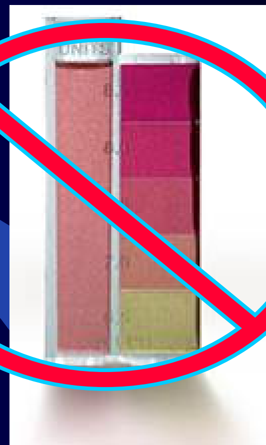
Sample Collection, Storage and Preservation

Generic Instruction

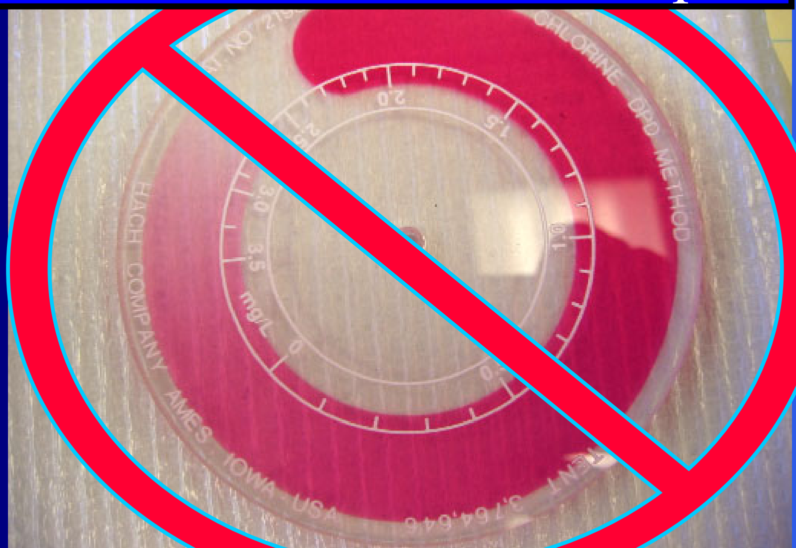
These instructions are simplistic. Alone, they are insufficient for proper testing.

Quick Tests for Chlorine Testing

- Colorwheels are NOT acceptable.
- "Pocket colorimeters" are NOT acceptable.
  - These colorimeters use an insufficient, preprogrammed calibration.
- Must use true spectrophotometers or colorimeters with a USEPA-accepted method.



These are NOT USEPA accepted



Procedure

Preparing Potassium Permanganate Standard

- In this test a potassim permanganate standard is used *instead* of a chlorine standard. Potassium permanganate is more stable than chlorine, and is used as a more reliable substitute standard.

Preparing Potassium Permanganate Standard

- Obtain <u>vendor prepared</u> potassium permanganate standard solution for use in DPD or FAS colorimetric methods.
  - Standard solution should be labeled 0.891g/l potassium permangantate. **This is equivalent to 1g/l (1000ppm) total residual chlorine**.
  - The standard solution should come in a dark amber glass bottle. This bottle should be stored in the dark, away from direct sunlight.

Preparing Potassium Permanganate Standard

CHLORINE STANDARD
(Potassium Permanganate, 0.891 g/l)
(For use in FAS colormetric test procedures)
Dilute 1 ml to 100 ml with distilled water to make a working solution. 1 ml working solution diluted to 100 ml = 0.1 ppm as Cl2.
Note: DPD Indicator must be added to your standards

WARNING — Not for internal use. Avoid contact with eyes, skin, or clothing. Use only as directed in test procedures. Keep out of the reach of children.

10117 NET CONTENTS 1 Pint

Standard is of correct concentration and labeled appropriately

Dark bottle prevents degradation due to light

Preparing Potassium Permanganate Standard

- Dilute the 1000 ppm stock solution to 1 ppm working solution (1:1000) as follows:
  1) Dilute 10.00 ml stock solution to 100.0 ml (with distilled water in a volumetric flask)
  2) Take 1.00 ml of this new solution and dilute it to 100.0 ml.
  – Be sure to mix each dilution thoroughly.

Remember:
The concentrations are equivalents. They solutions are not ACTUALLY chlorine, but behave like chlorine at the equivalent concentration.

Preparing Calibration Standards

- Fresh working solution for use in calibration standards must be prepared fresh for that day.
- Range of calibration standards must include the anticipated sample range.
  - If you expect samples between 0.030 – 0.150 ppm (typical for wastewater and drinking water analysis), then calibrate from 0.020 – 0.200 ppm.
- Do not prepare calibration standards below 0.020 ppm. These will lead to error.
- Set up a table to help calculate dilutions

Preparing Calibration Standards

- Example dilution table.

  Dilute working solution (1ppm) with DI water up to the final volume in a 100 ml volumetric flask:

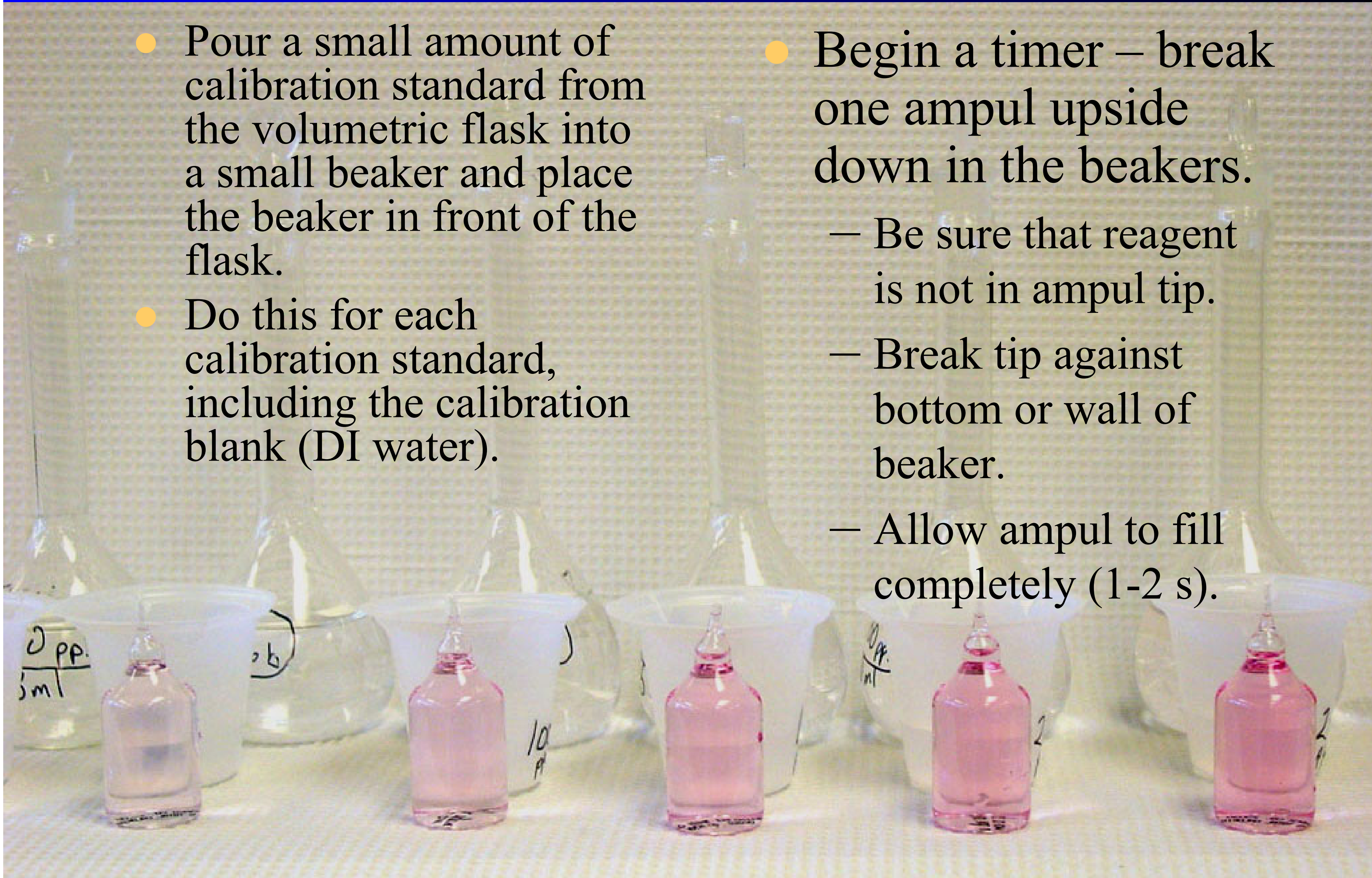
Working Solution 1ppm	Final Volume	Final Concentration
3.00 ml	100 ml	0.03 ppm
5.00 ml	100 ml	0.05 ppm
7.00 ml	100 ml	0.07 ppm
10.00 ml	100 ml	0.10 ppm
20.00 ml	100 ml	0.20 ppm

Preparing Calibration Standards

- ✓ Calibration standards are labeled, filled to meniscus, and thoroughly mixed.
- ✓ Calibration blank (DI water) is included, and prepared like all other standards.

Note: this photograph reflects concentrations in ppb, not ppm

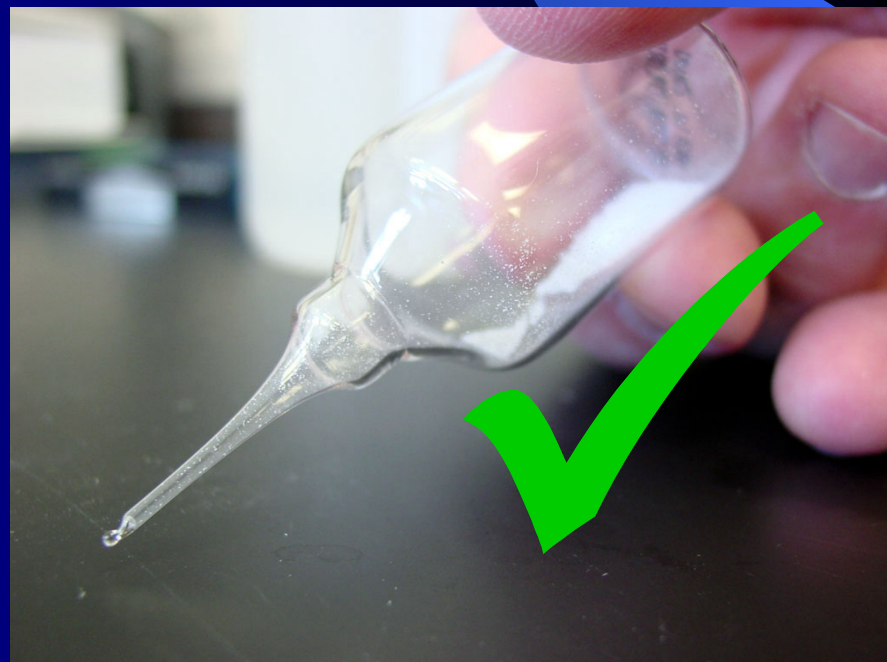
Developing Calibration Standards



- Pour a small amount of calibration standard from the volumetric flask into a small beaker and place the beaker in front of the flask.
- Do this for each calibration standard, including the calibration blank (DI water).
- Begin a timer – break one ampul upside down in the beakers.
  - Be sure that reagent is not in ampul tip.
  - Break tip against bottom or wall of beaker.
  - Allow ampul to fill completely (1-2 s).

How to Break an Ampul

- Reagent is in tip of ampul.
- Reagent is likely stay inside the broken tip after it breaks, increasing chance for low bias.

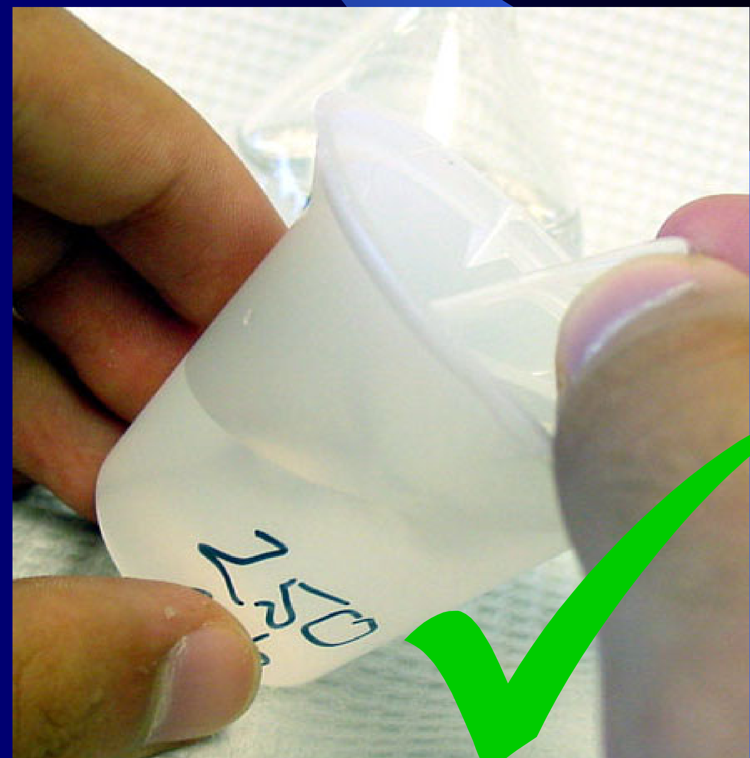


- Ampul is handled carefully to avoid reagent falling into tip.

How to Break an Ampul

- Ampul is too vertical.
- Reagent is likely to fall into tip before it breaks.

- Beaker and ampul are tipped to the side.
- Reagent is more likely to stay in the ampul and not fall into tip.



DPD Color Levels

“Typical”
chlorine sample
- light magenta

“Strong”
chlorine sample
- deep magenta

“Overshot”
chlorine sample
- reddish brown

Destroyed DPD
- colorless
(pure chlorine
bleach)

Making a Calibration Curve
Zeroing the Spectrophotometer

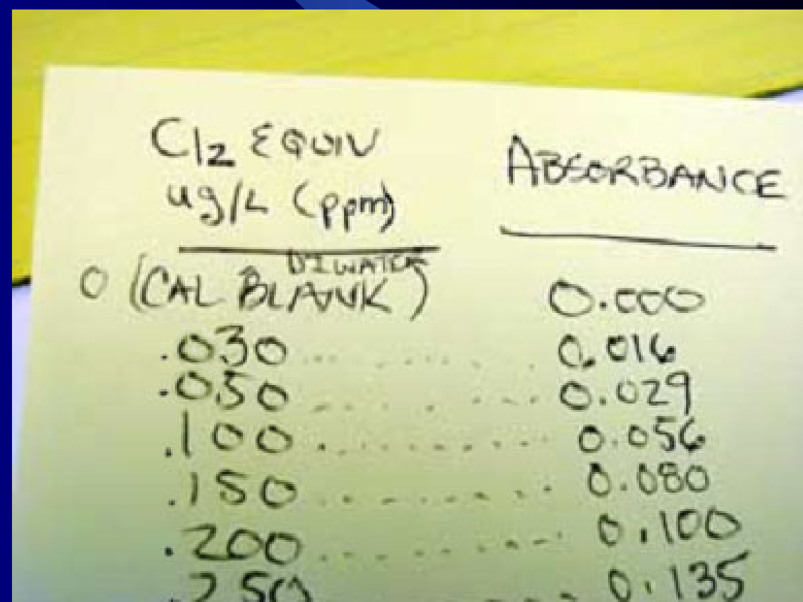
- Zero the spectrophotometer using the calibration blank, DI water
  - Make sure the spectrophotometer is set to 515 nm. If this is not possible, use a wavelength between 515-540 nm.
  - Zero with the same water that was used in creating the calibration standards.
  - Pour the water from the flask into a clean sample cell. Wipe the cell clean of fingerprints.
  - Align the reference mark to the light source (or as appropriate), and zero the spectrophotometer.

Fingerprints, dust, and other residues can bias absorbance readings. Be sure to cleanly wipe off the optical surface of any sample cell or ampul that is analyzed with the spectrophotometer.

Making a Calibration Curve
Zeroing the Spectrometer

Making a Calibration Curve: Record Data

- Record the absorbance of each calibration standard
- This data is used when calculating a calibration curve.



Making a Calibration Curve: Determining r (correlation coefficient)

- Use a calculator, Excel, or other software.
- In Excel, you can use the CORREL formula.
- CORREL($Values_1$,$Values_2$) returns the correlation coefficient, r.
- Many scientific calculators can calculate r as well.

0	0.000	
30	0.016	
50	0.029	
100	0.056	
150	0.080	
200	0.100	
250	0.135	
r =	0.998079	
=CORREL(A1:A7,B1:B7)		

Making a Calibration Curve: About r

- r describes how much of a correlation there is between the two data sets.
  Click here for more information.
- In this case, it describes how *linear* the data is.
- Why should the data be linear?
- Because of Beer's law.

Making a Calibration Curve: Beer's Law

- Beer's law states that

  $$A = \varepsilon b c$$

  ε = molar absorptivity (how "potent" the dye is)

  b = cell path length

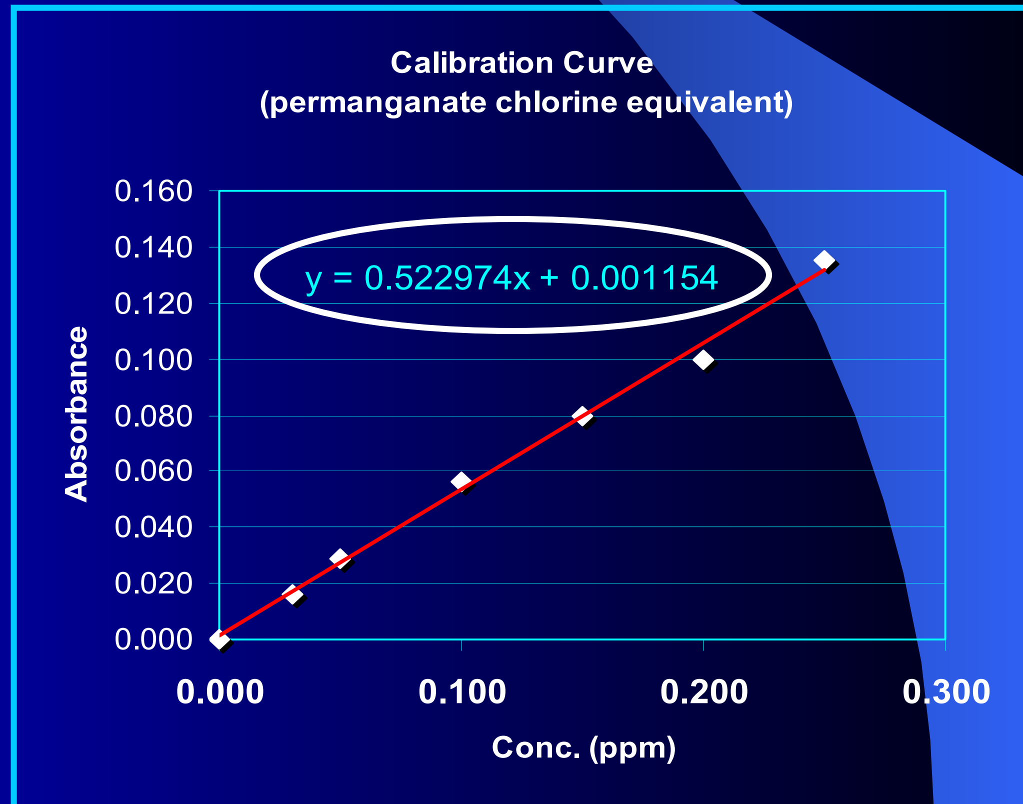
  c = concentration

- Since the molar absorptivity and the path length are the same for all readings, only the the concentration changes. Thus, if the concentration doubles, so should the absorbance (**A**). This is a ***linear*** relationship.
- If calibration data is not linear ($r < .995$), than there is a problem in either the procedure or calibration stsandard.

Making a Calibration Curve: Linear Regression

- Once you know that the data is valid (r > .995), you will need to calculate the linear regression, or the “line of best fit”.
- This can be done on a calculator or other software.

0	0.000
0.030	0.016
0.050	0.029
0.100	0.056
0.150	0.080
0.200	0.100
0.250	0.135
r =	0.998079
=CORREL(A1:A7,B1:	



Making a Calibration Curve: Check Calibration

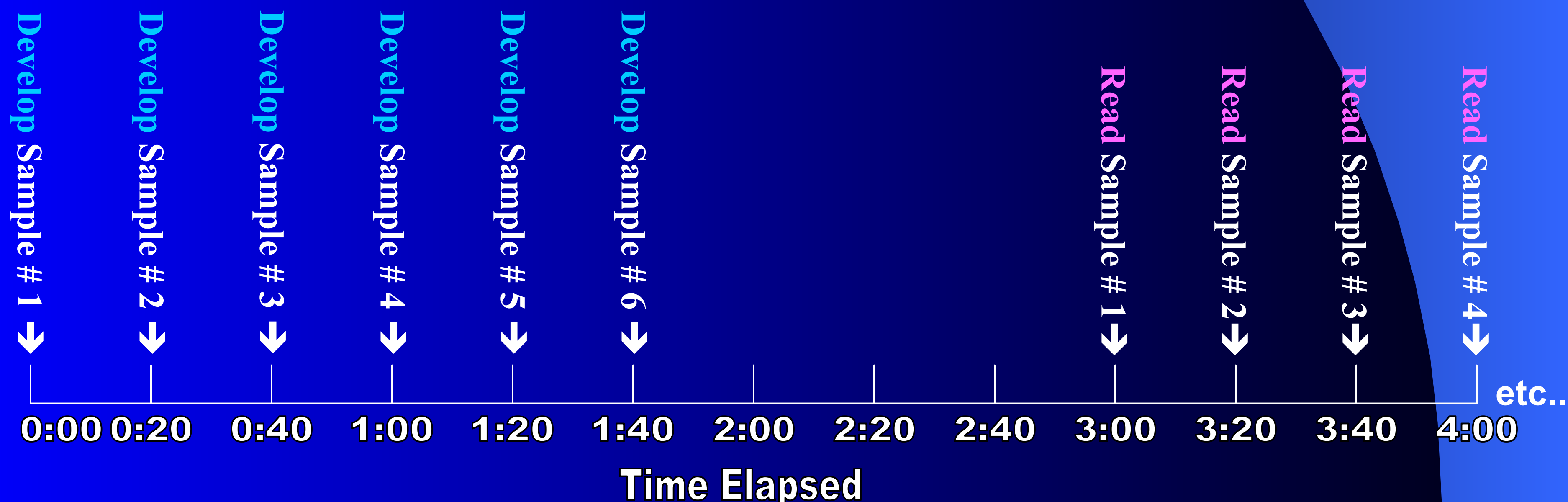
- Calibration is valid when $r > .995$ and the y-intercept (the “b” in $y=mx+b$) is below the limit of detection (LOD).
- There is extensive calibration information on available in other DNR presentations. Check it out!

Analyzing a Sample

- There are three steps to analyzing a sample.
  - Zero the spectrometer with DI water
  - Record absorbance of blank (pure sample)
  - Record absorbance of developed sample (sample + DPD in ampul).

Analyzing a Sample - Timing

- Developed (solution + DPD) samples or standards should not be read before three minutes, or after six minutes. Read them between 3-6 minutes.
- Set up a timing schedule to maximize efficiency.
- Ensure that each sample develops for a consistent amount of time before it is read.



Analyzing a Sample

- When analyzing a sample record both absorbances (blank and developed) on the data sheet.
- Subtract the blank absorbance from the developed absorbance to get the adjusted absorbance.
- This adjusted absorbance corrects for any natural absorbance of the sample or ampul itself.

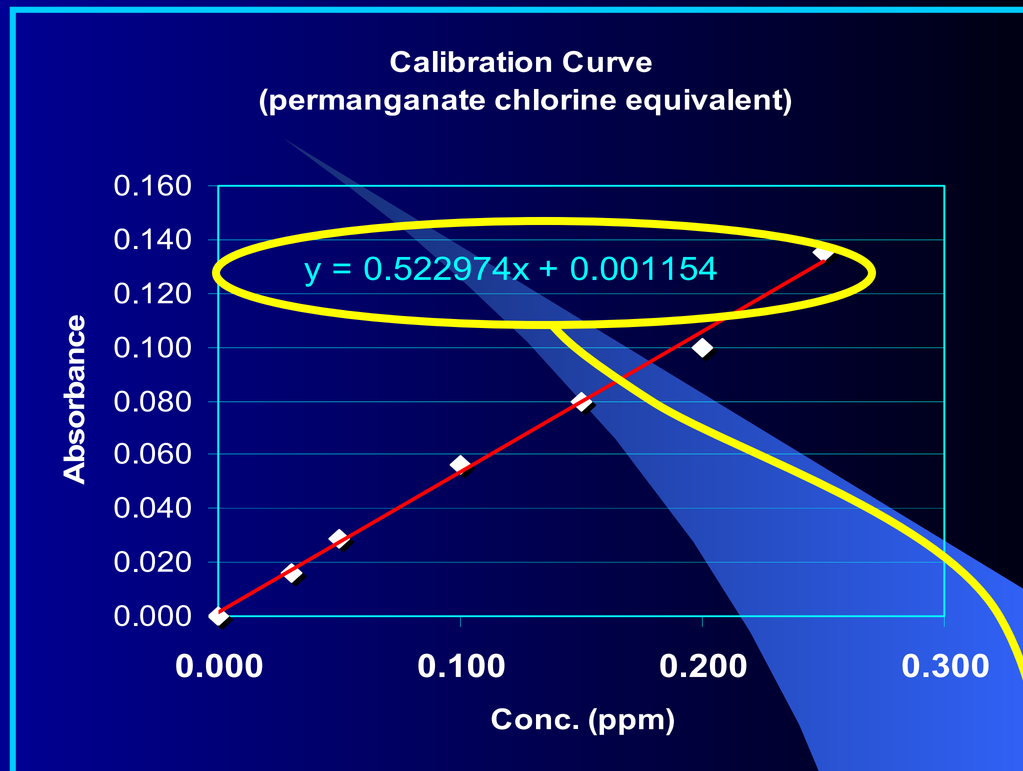
Example

Sample no.	Blank	Developed	Adj. Abs.
Outfall no. 1	0.003	0.032	0.029
Outfall no. 2	0.003	0.048	0.045
Outfall no. 3	0.000	0.046	0.046
Outfall no. 4	0.002	0.035	0.033

DEVELOPED - BLANK = Adjusted Absorbance

Analyzing a Sample

- The adjusted absorbances are the only values that are used in calculations.
- These values are "plugged" into the linear regression equation to get a concentration.



Example

- Let say, for instance, that a sample has a corrected absorbance of .065. Use back-calculation to calculate the concentration of sample, using the linear regression equation. Solve for (x).

[Corrected Abs.] = .522974(conc. of sample) +.01154

[.065] = .522974(x) +.01154 **x=.102 ppm**

Summary

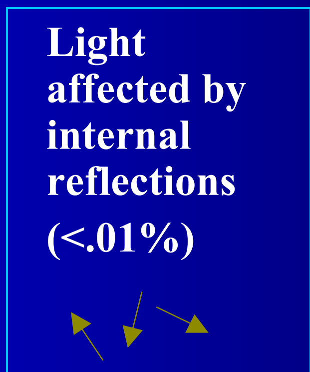
- Make sure to use an USEPA accepted method, and be able to cite which method that is.
- Be sure to calibrate carefully. Check that calibration is valid (>.995).
- Determine line of best fit.
- Use equation from line of best fit to back-calculate absorbances from samples.

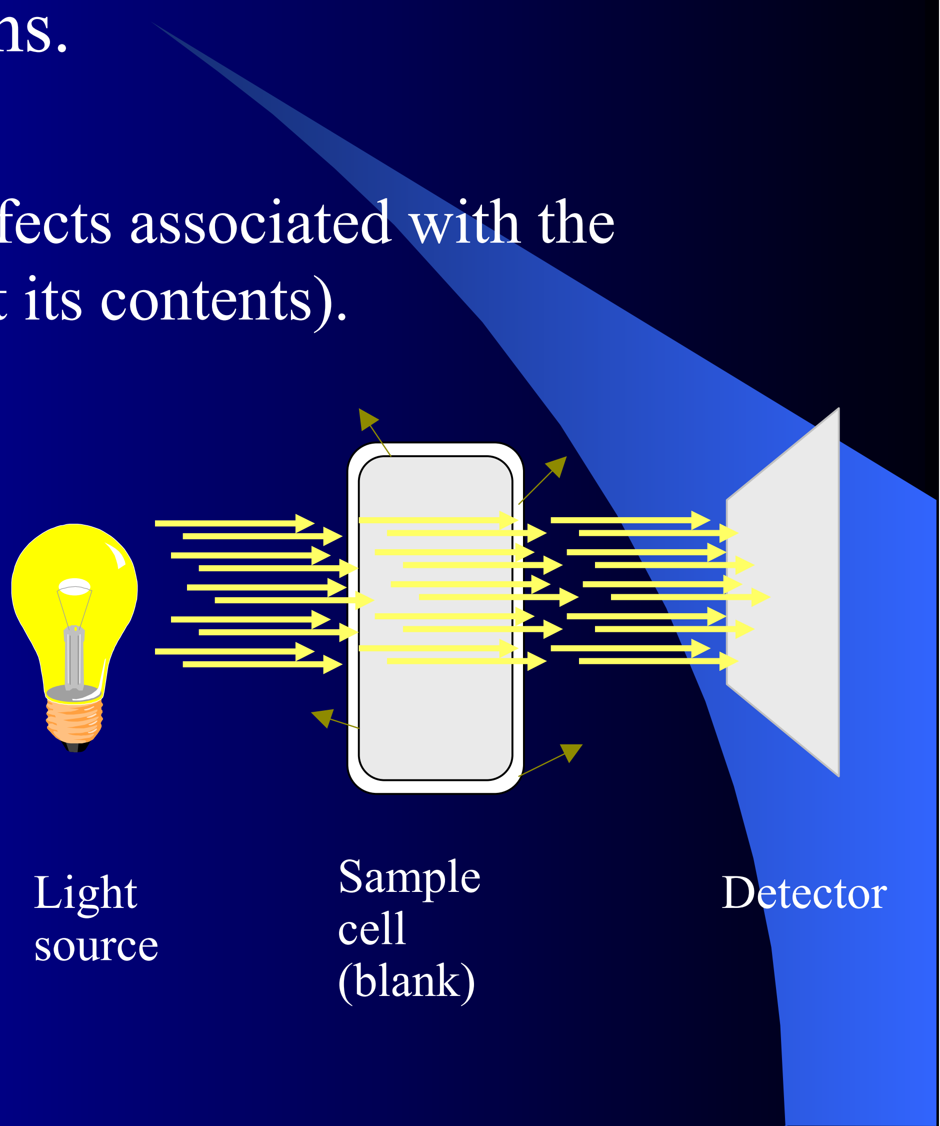
Good Luck!

Additional Information

About Low Level Testing

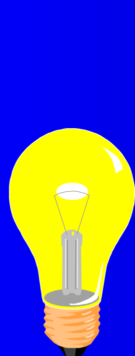
- Low level testing is especially prone to the effects of internal reflections.
- Internal reflections:
  - light-scattering/reflecting affects associated with the sample cell/ampul itself (not its contents).
- These effects are usually insignificant.

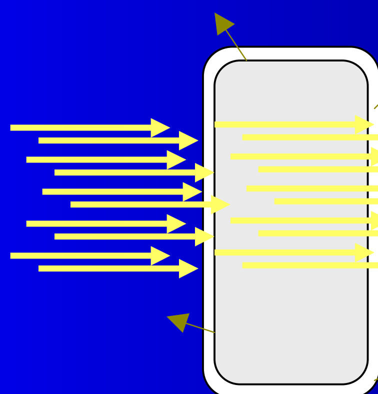




Typical Spectrophotometry

- Ideally, there is a significant difference between the blank and sample. The random differences in scattering effects of different cells is insignificant.

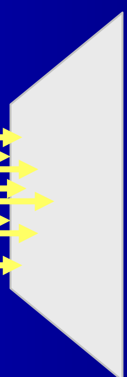


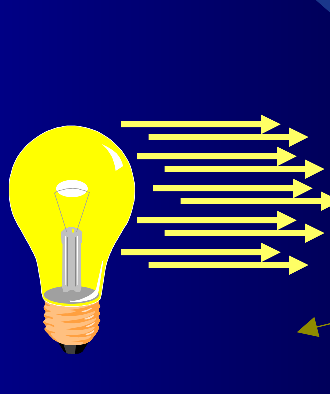


Strong signal

Typical Blank

- Little absorbance
- Most of the light goes through





Weaker Signal

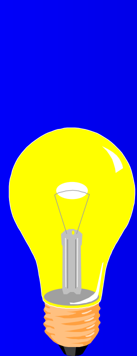
Typical (developed) Sample

- Significant Absorbance
- A significant amount of light is absorbed

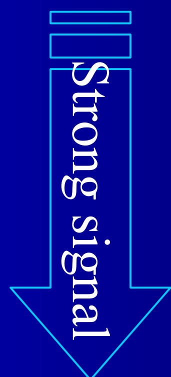
- Absorbance = Strong - Weak = **strong**
- Uncertainty due to different internal reflections is insignificant

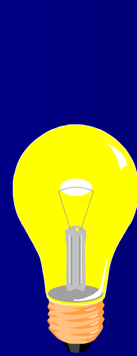
Low-Level Spectrophotometry

- The blank and the sample are nearly identical
- Differnces between cells become significant.



Blank
- Little absorbance
- Most of the light goes through





Low absorbance sample
- Little absorbance
- Most of the light goes through

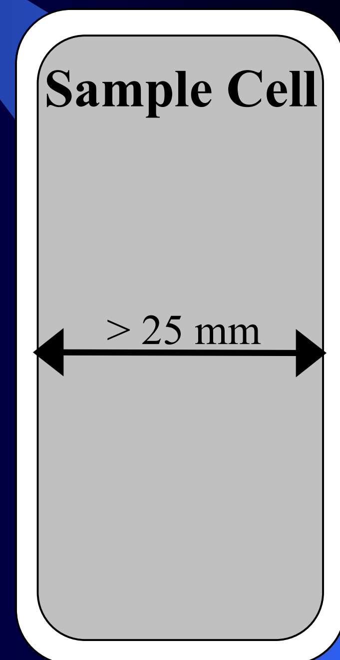
- Absorbance = Strong - Strong = ~ 0
- Uncertainty due to different internal reflections is more significant

Low-Level Spectrophotometry: Minimizing Internal Reflections

- Use reagent-free ampuls for blank and/or zero
  - Blank ampuls are identical to DPD ampuls, but contain no DPD reagent
  - Minimizes the differences in internal reflections between readings
  - This is somewhat more costly (~ 50 cents per blank ampul) but will improve results.

Low-Level Spectrophotometry: Minimizing Internal Reflections

- Use cells that w/ 25mm+ pathlength
  - Cells smaller than 25mm across will yield low absorbances. Internal reflections will be more significant.
- Avoid diluting sample
  - Diluting the sample will further decrease its absorbance.
- Use flow-through cell to *eliminate* errors due to internal reflection.
  - Costly, but significantly improves results.



About r

- Let’s look at the men of Happyville. Data was collected concerning height and weight. It is shown below.

Can you predict the weight of a 5’9” person from this data?

Height	Average Weight
5' 2"	142.1
5" 4"	147.7
5' 6"	156.0
5' 8"	164.3
5' 10"	172.1
6' 0"	179.6
6' 2"	188.1
6' 4"	199.0

About r

- Can you predict the weight of a 5’9” person from this data?

Sure, the data is predictable and is very linear. By drawing a line through the points on a graph, we can predict the weight of the average 5’9” male. **168.4**

Height	Average Weight
5' 2"	142.1
5" 4"	147.7
5' 6"	156.0
5' 8"	164.3
5' 10"	172.1
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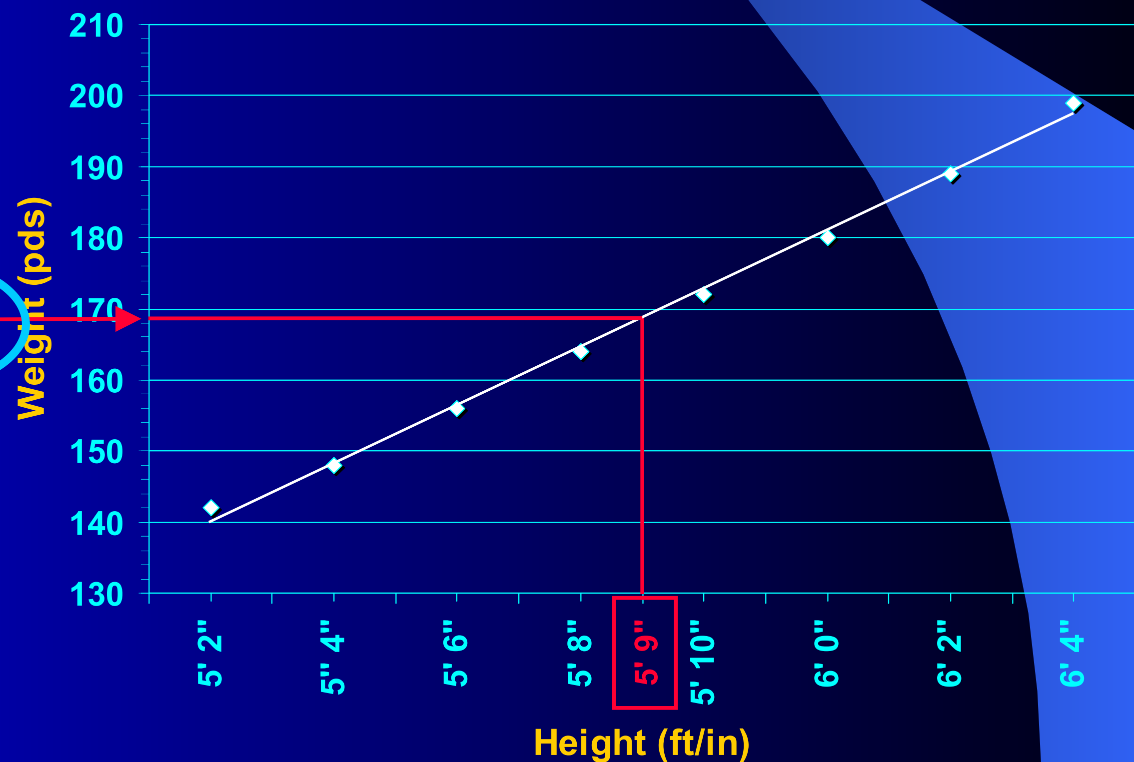
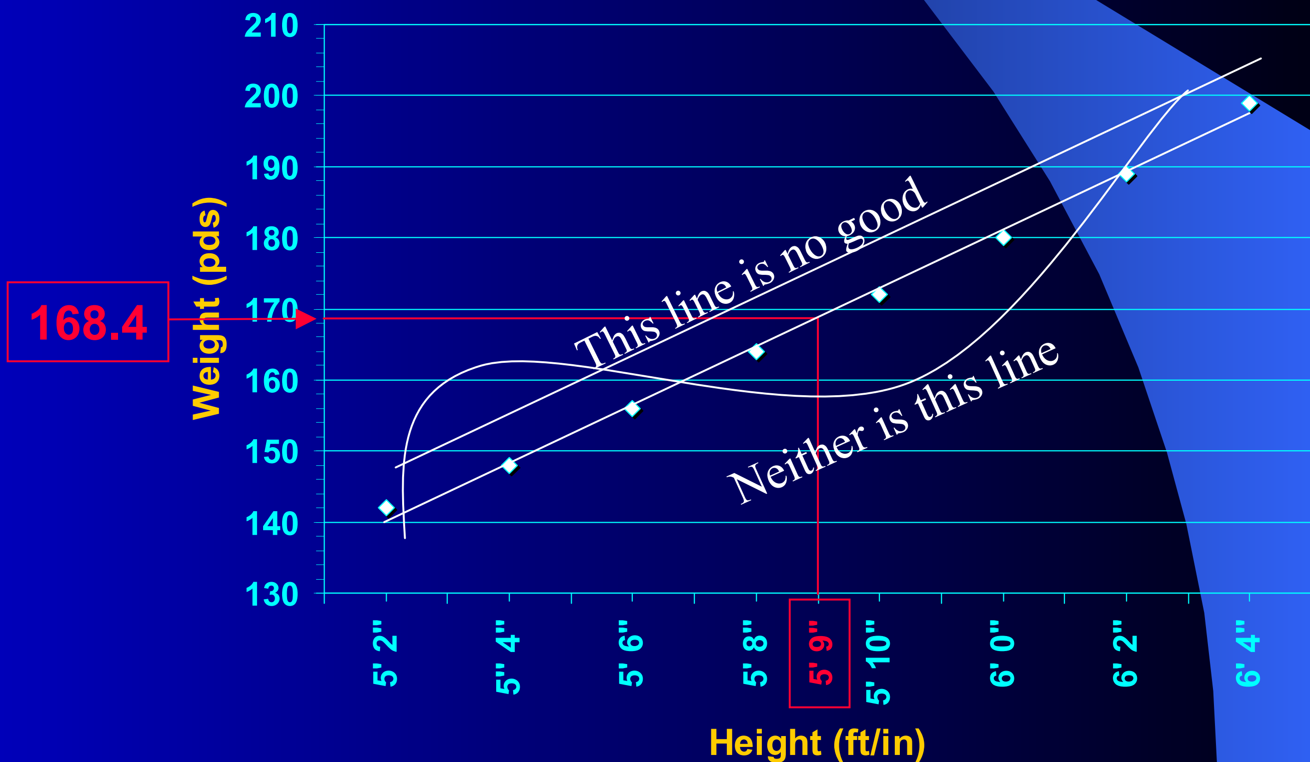


About r

- We can assume that the line we drew through the points is a good indicator of reality because it lies close to every point. r describes how well the line describes the true data (reality).

Height	Average Weight
5' 2"	142
5" 4"	148
5' 6"	156
5' 8"	164
5' 10"	172
6' 0"	180
6' 2"	189
6' 4"	199



About r

- Let’s look at some other data. This time we will collect data on the number of children men in Happyville have. The results are shown below
- Can you predict how many children the average 5’9” man has?

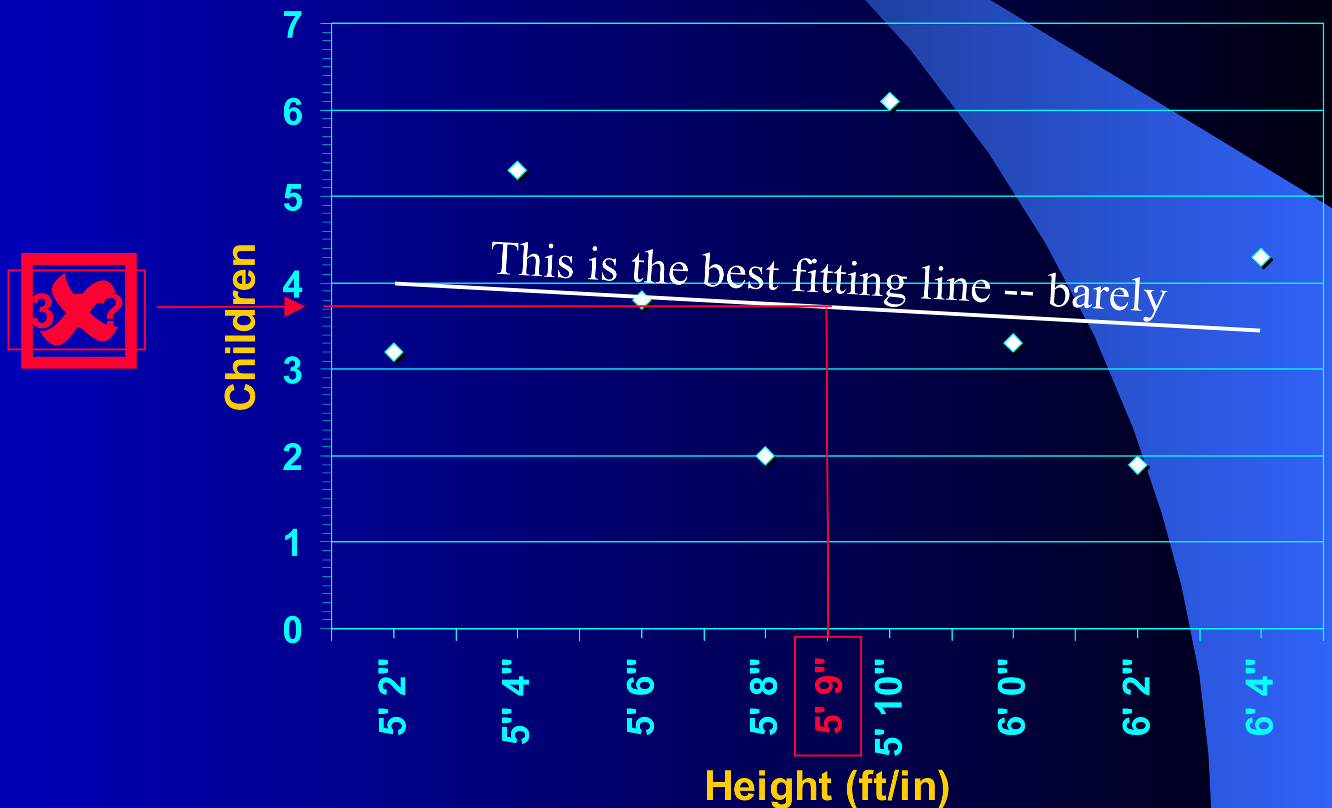
Height	Average # of Children
5' 2"	3.2
5" 4"	5.3
5' 6"	3.8
5' 8"	2.0
5' 10"	6.1
6' 0"	3.3
6' 2"	1.9
6' 4"	4.3

About r

- Can you predict how many children the average 5’9” man has?

Probably not, the data is unpredictable because it is not linear. In fact it is not ordered at all. It would be difficult finding ANY line that comes close to all the points.

Height	Average # of Children
5' 2"	3.2
5" 4"	5.3
5' 6"	3.8
5' 8"	2.0
5' 10"	6.1
6' 0"	3.3
6' 2"	1.9
6' 4"	4.3

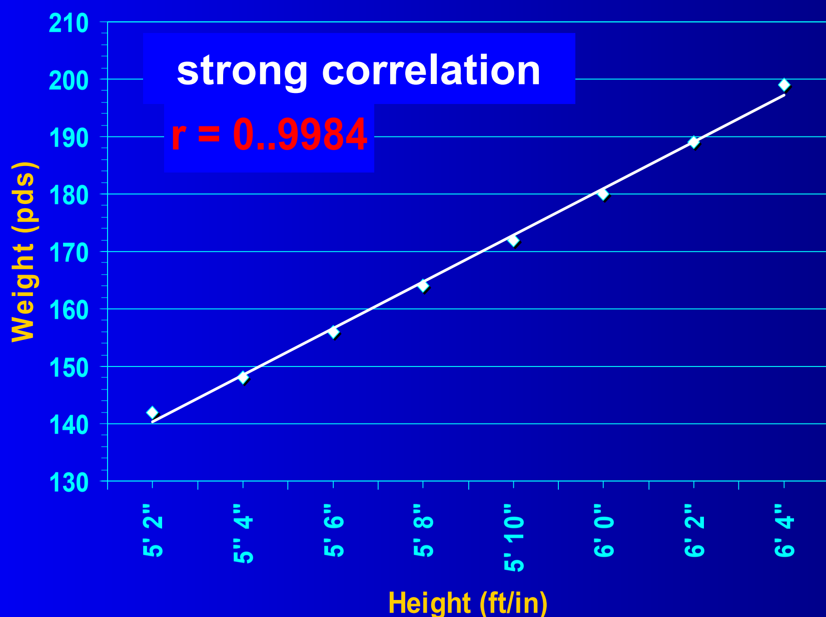


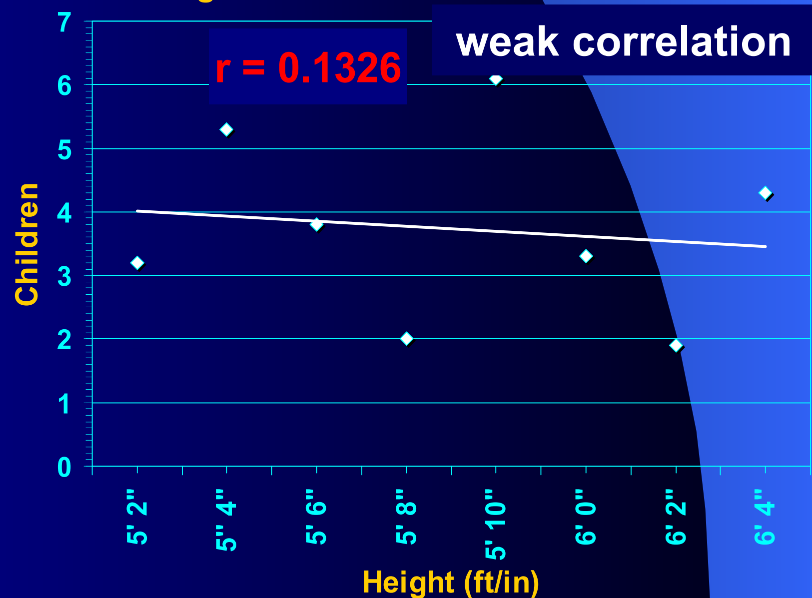
About r

The ability for a line to closely fit the data is its correlation coefficient, or r value. r is valued between 0 and 1, 0 meaning NO correlation and 1 meaning perfect correlation. Most scientifically data falls between the two.

Average Weight	Height	Average # of Children
142	5' 2"	3.2
148	5" 4"	5.3
156	5' 6"	3.8
164	5' 8"	2.0
172	5' 10"	6.1
180	6' 0"	3.3
189	6' 2"	1.9
199	6' 4"	4.3

Thus, we can say that there IS a correlation between height and weight, but there is NOT a correlation between height and no. of children.





About r

- So what does this have to do with chemistry?
- The amount of Würster dye produced from the DPD reagent is directly proportional to the amount of chlorine in the sample. The colored dye is responsible for the absorbance at 520nm.
- Thus Beer's law tells us that the absorbance of a sample with excess DPD will be directly proportional to the amount of chlorine in it.
- We use r to check how well Beer's law is working under specific laboratory conditions.