Chemistry and Instrumentation

By: Aaron Golston
Quality Assurance Manager
Lagunitas Brewing Company
Chicago, IL

Dangerous Words and Practices

- We have always done it this way.
  - Doesn't mean it can't be improved upon or made more efficient.

- SOPs not documented or formally trained
  - SOPs in place, dynamic and in control.
  - Variations in procedure can lead to error

- Make no assumptions about instruments or calibrations
  - Validate calibrations before use by running control samples
  - Establish acceptable limits for controls
  - Have reaction procedures for out of specification control samples

Terpenes

IPA

Diacetyl

Crystal Malt

Foam

Esters

Wheat

Real Extract

Alcohol

pH

Turbidity

Calcium

Yeast

Water

Ale

Head

Zinc

Lager

Pale Malt

Color

Hops

Sours

Bitterness Units

Iron

Polyphenols

Enzymes

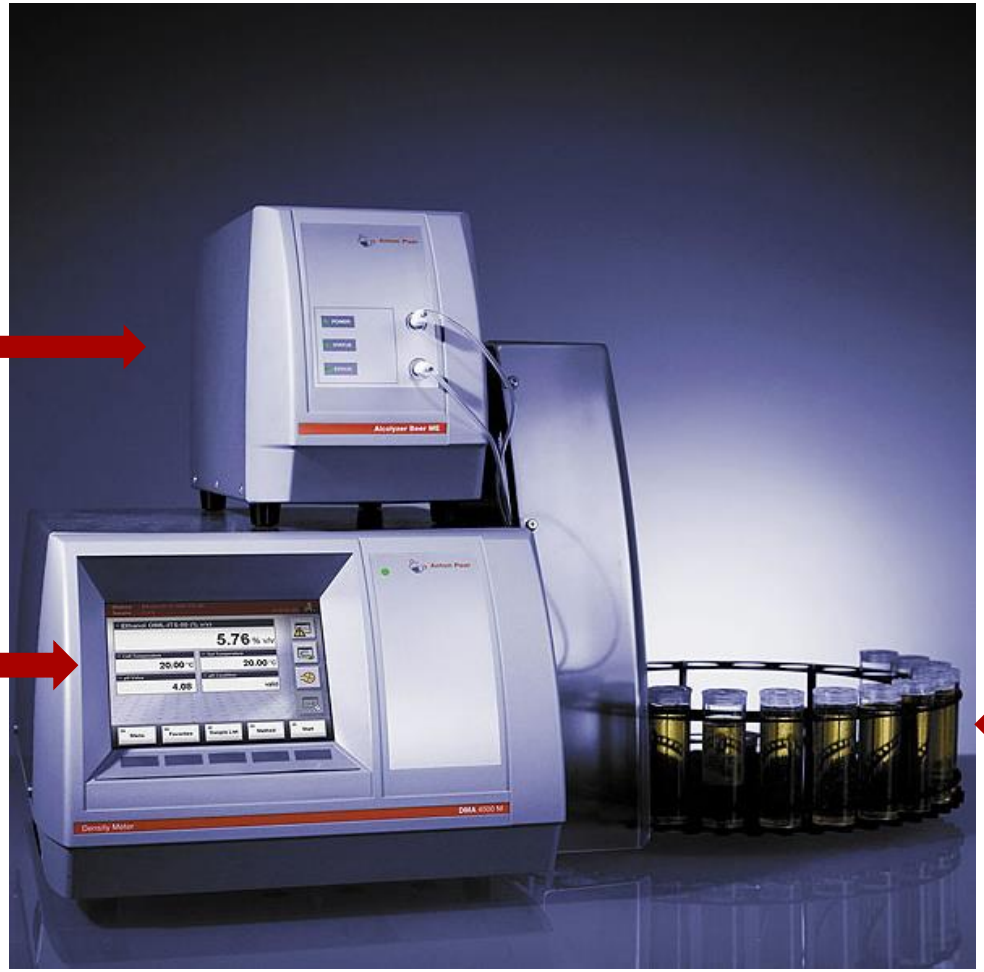
FAN

Quick Overview

- Alcohol and residual extract analysis
- pH analysis
- Bitterness analysis
- Color analysis

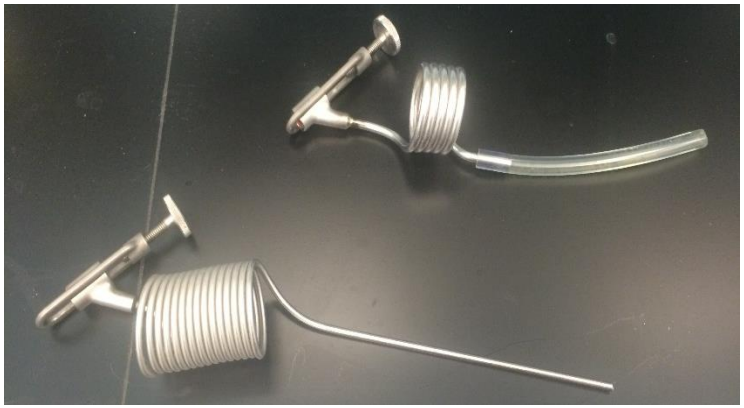
- Side note: Best when users understand how the instruments work.

Alcohol and Residual Extract Analysis



Alcohol and Residual Extract Analysis – Sampling

- Alcohol analysis is very simple but easy to make little mistakes
  - Open ports or zwickles
    - Ethanol is volatile and can evaporate during sampling if the fill isn't gentle, especially during fermentation when the sample is warm
  - Pigtails
    - Give gentle sample container fills that minimizes foaming and ethanol loss due to evaporation.
    - Large Bore
      - Good for sampling in fermentation when there are low levels of carbonation in the beer and higher levels of solids. They are less likely to clog with hops or yeast.
    - Small Bore
      - Good for sampling when the beer is carbonated or under pressure and lower levels of solids. It will take longer to fill the sample container than the large bore but will save time when compared to pulling rechecks of questionable values.
  - Bottle Rinsing
    - Any residual water left in the bottle will artificially decrease all values the Alcolyzer will generate



Alcohol and Residual Extract Analysis – Sample Prep

- Sample preparation
  - ASBC Method
    - Brewhouse
      - Lots of particulate
    - Fermentation
      - Remove yeast, hops, other particulate and decarbonate
    - Brite beer / packaged beer
      - Decarbonate
- Why?
  - Alcolyzer is an optical instrument. Designed to handle clear, brite liquids with low levels of suspended particulate.
  - Extremely hazy or turbid beers can cause artificially low values.

Alcohol and Residual Extract Analysis - Results

- The results you get are only representative of what was in the sample bottle. The results can be extrapolated back to what is in the bulk tank, assuming the contents is homogeneous.
- What can the results be used for?
  - SO MUCH MORE THAN ALCOHOL!
  - Daily Fermentation Monitoring
  - End of Fermentation Analysis
    - Alcohol
      - Ensures you won't drop illegally low during filtration
    - Real Extract
      - Makes sure your beers body is consistent and you aren't wasting malt trying to hit ABV when you could be adjusting your process!
        - » As you grow, purchase instruments that can be used to optimize the process.
  - Brite Beer Release
  - Package Sample Analysis

Alcohol and Residual Extract Analysis - Results

- Instrument cost ~$50K
- Example:
  - 50 bbls of 15°P with 78.8% CGE, As is and 85% efficient brew house costs @ $0.58/lbs

$$50\ bbls * \frac{117.34\ L}{1\ bbls} * \frac{0.15\ kg\ extract}{1\ kg\ water} = 880.1\ kg\ \text{extract} * \frac{1}{78.8\%\ CGE} * \frac{1}{85\%\ BH\ Efficiency} * \frac{2.2\ lbs}{1\ kg} * \frac{\$0.58}{lbs} = \$1{,}676.54$$

  - 50 bbls of 15.2°P with 78.8% CGE, As is and 85% BH efficiency costs @$0.58/lbs

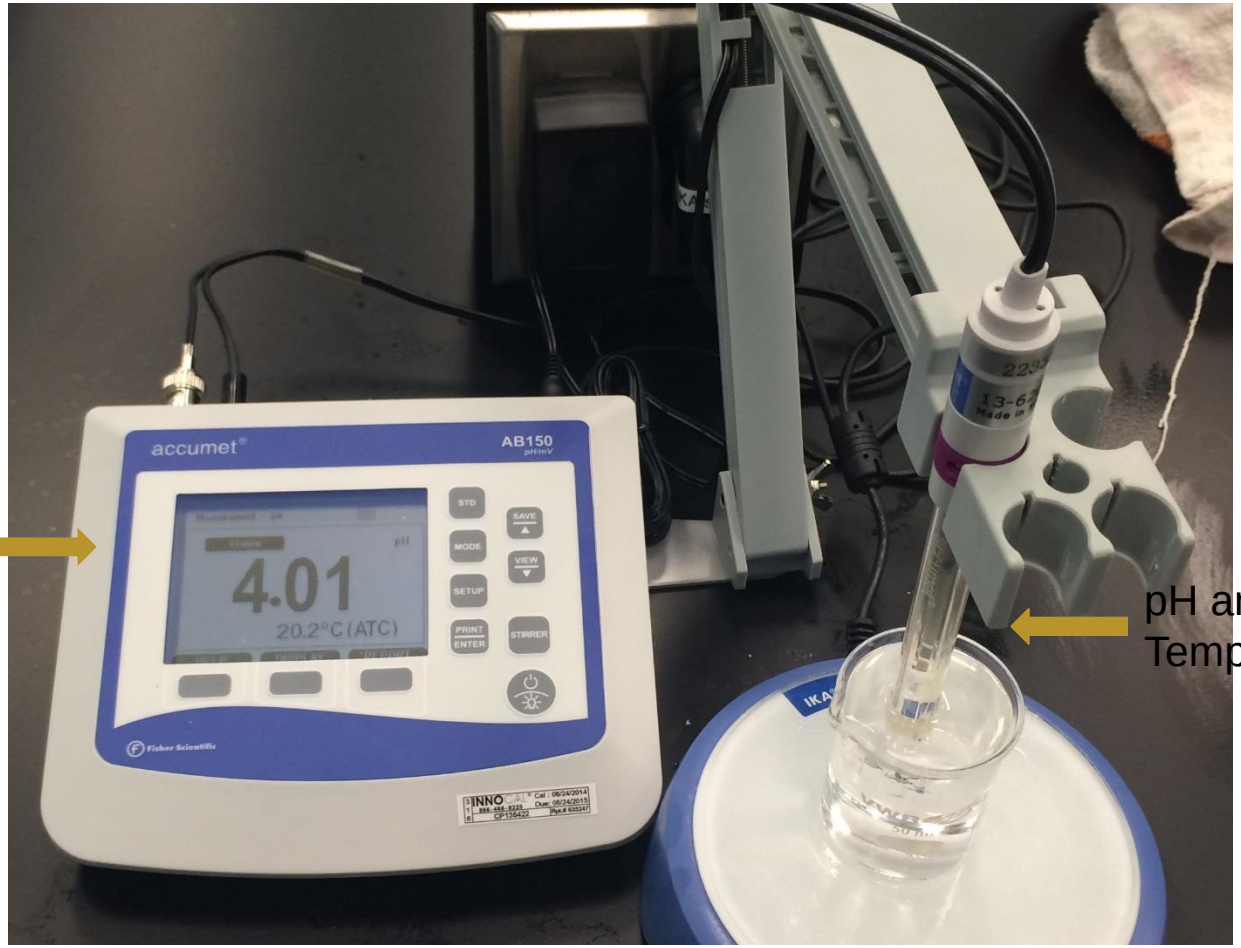
$$50\ bbls * \frac{117.34\ L}{1\ bbls} * \frac{0.152\ kg\ extract}{1\ kg\ water} = 891.8\ kg\ \text{extract} * \frac{1}{78.8\%\ CGE} * \frac{1}{85\%\ BH\ Efficiency} * \frac{2.2\ lbs}{1\ kg} * \frac{\$0.58}{lbs} = \$1698.92$$

  - Only $22.38 a brew more!
    - 5,000 bbls = 100 brews = $2,238
      - 25 year Return on Investment (ROI)
    - 20,000 bbls = 400 brews = $8,952
      - 6.5 year ROI
    - 50,000 bbls = 1,000 brews = $22,380

Alcohol and Residual Extract Analysis – Summary

- Only as good as your calibration and sample preparation
  - Validate the DMA is reading correctly on water
  - Validate the Alcolyzer is properly calibrated using a known standard
  - Develop consistent sampling practices
  - Develop consistent sample preparation procedures.
  - DOCUMENT EVERYTHING!!!
  - TRAIN EVERYONE!!!!
- Challenging yourself and your procedures
  - BAPS or ASBC
  - Outside lab analysis
  - ABV by GC (TTB Method)

pH Measurement



pH Measurement – Sampling, Sample Prep and Analysis

- As with Alcohol measurement, gentle filling of sample containers with a pigtail is best to ensure consistency between technicians.
- Degassing is a MUST!!
  - CO_2 dissolved in solution suppresses the pH due to the formation of carbonic acid.

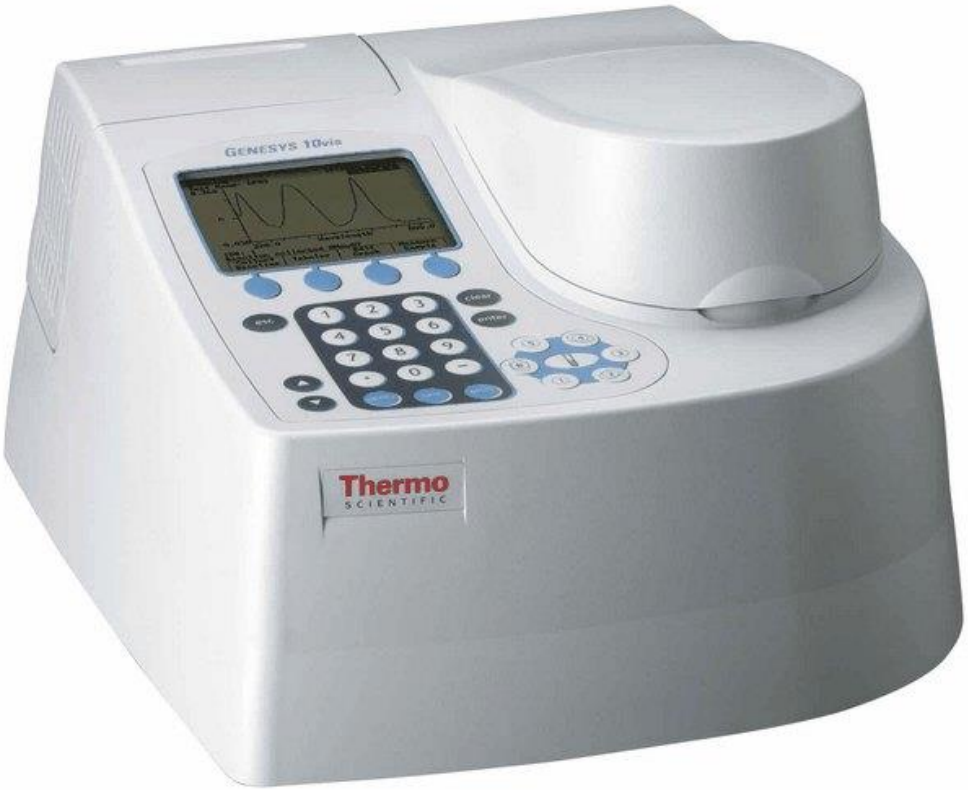
$$H_2O + CO_2 \rightleftarrows H_2CO_3 \rightleftarrows HCO_3^- + H^+$$

  - Centrifugation, warm sample to room temperature and then filtration or shaking
    - Whatever you do…utilize a consistent method
- Ensure the sample is homogeneous.
  - Use a stir bar to mix sample during measurement.
- Rinse and dry probe between measurements
- Store in appropriate buffer solution

pH Measurement - Interpretation

- pH measurement can be used for many things and be very telling about the process.
  - Mash monitoring and optimization
    - pH is ideal for enzyme performance and additions are correct
  - Kettle monitoring
  - Whirlpool monitoring
    - pH is ideal for finings
  - Yeast monitoring
    - Yeast harvests are healthy and have low pH.
      - Higher pH can indicated stress or poor health.
  - Fermentation monitoring
    - Yeast are healthy and producing expected amount of acid.
  - End of Fermentation Analysis
    - Completed fermentation and crashing, this is the final check before filtration
  - Brite Beer Analysis
    - Make sure nothing happened between fermentor and BBT fill.
      - Increase or decrease in pH could indicate cleaning chemical contamination
  - Package Sample Analysis
    - Make sure nothing happened between BBT and package filling.
      - Increase or decrease in pH could indicate cleaning chemical contamination.

BU and Color Measurement



BU and Color Measurement – Sampling

- Iso-α-acids are foam-positive. Sample with a pigtail and gently fill sample container.
  - High BU, high ABV beers, this will be less of an issue as 2 – 5 BUs will likely not jeopardize the taste or stability of the product.
  - Low BU, low ABV beers, this is a HUGE issue as this level of variance can be close to the low end of where iso-α-acids inhibit microbial growth.

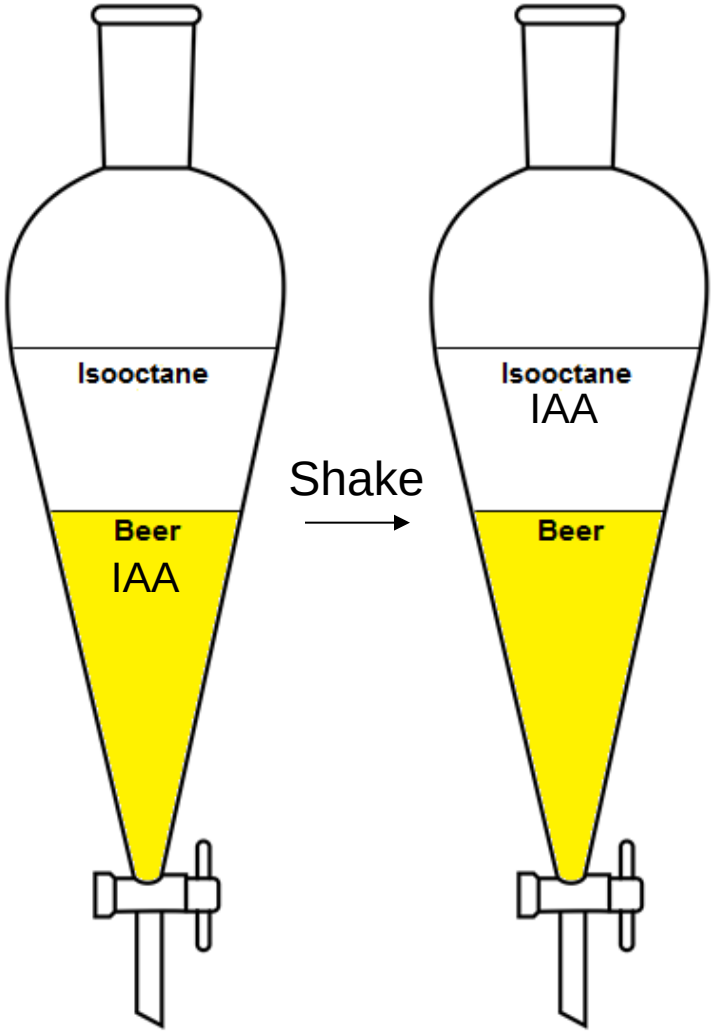
- Color isn't affected by the way the sample is collected but the clarity of the sample can be. A spectrophotometer is a precision optical instrument so the sample must be clear to allow to accurate measurement.
  - Haze from the use of wheat or other adjuncts can cause haze to be high which will affect to color.
  - Foam can collapse back into the beer causing the beer to be hazy which will affect the color result. Poor transmittance of "light" will increase the color values artificially.

BU and Color Measurement – Sample Prep

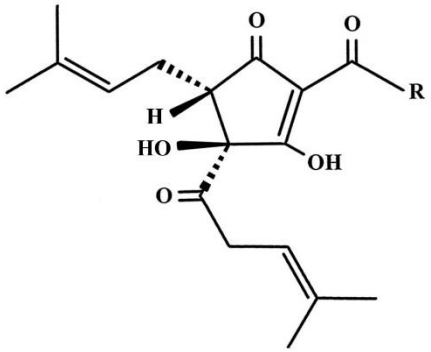
- BU samples should be transferred with no foam
  - Keep samples cold before analysis
    - OK to freeze
  - OK to warm samples after transfer to extraction tube to avoid emulsions

- Color samples must be bright for analysis
  - Centrifuge, filter or syringe filter to clarify the beer
  - Yeast, pentosan haze, hop particles or other suspended solids can cause issues if not removed.
  - Preparation method needed should be dictated by your specific process

Bitterness Units Extraction

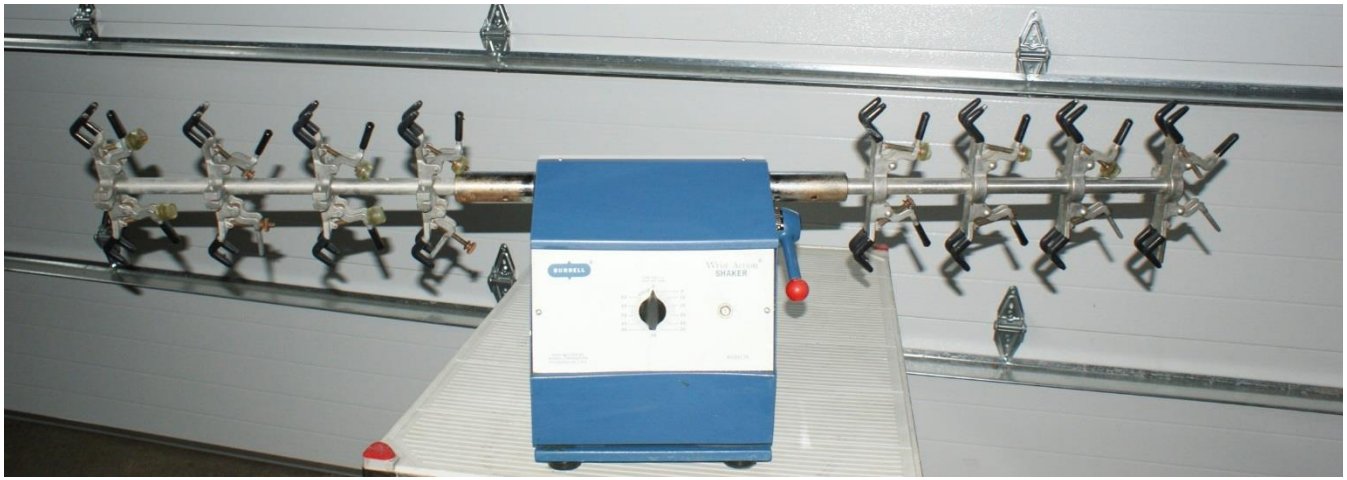


- Liquid / Liquid Extraction
  - Organic Layer: UV grade Isooctane
  - Aqueous Layer: Beer
- Target Analyte
  - Iso-α-acids (see below)
    - Aqueous phase is acidified to drop pH below the pKa of the IAA. This ensures all the carboxylic acid functional groups are protonated making the IAAs less polar. This change in polarity increases the IAA solubility in the isooctane (non-polar phase)
    - Other compounds such as polyphenols, α-acids and hop oxidation products are also soluble.
    - 1 BU ≠ 1 ppm



BU Measurement - Extraction

- Vigorous mixing is requisite!
  - Layers aren't miscible but there is transfer between them, which is increased by increased surface area contact between the two immiscible phases.
  - Mount clamps vertically
  - Visually observe and test all positions for consistency



BU Measurement - Extraction

- If the sample has emulsified and cannot be separated by centrifugation, perform the test again.
  - Small amounts of emulsions are ok, ~ few mLs
- Isooctane is volatile and samples should be measured as soon as the extraction is complete.
  - Don’t transfer to the cuvettes and let sit before measuring.

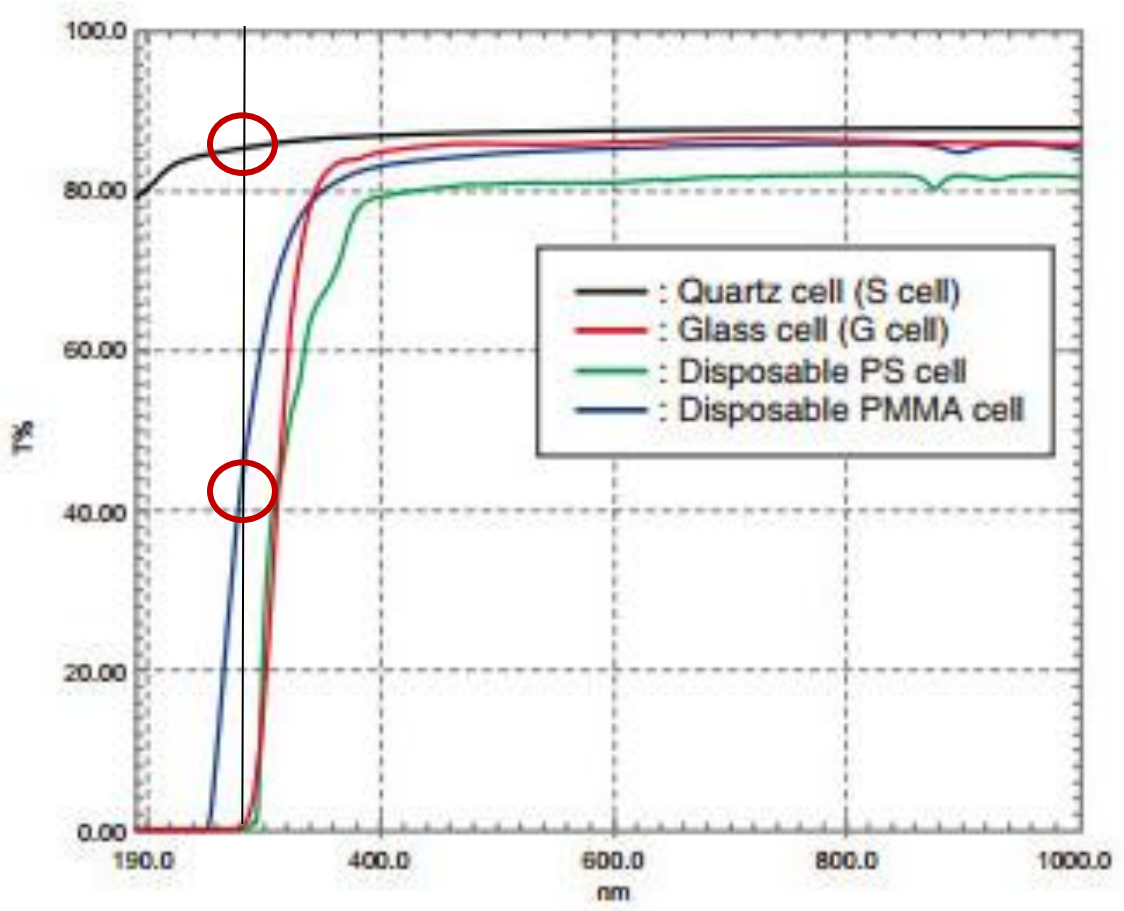
Cuvette Comparison

Bitterness Units

- BU @ 275 nm, UV spectrum
- Quartz cuvettes transmit ~85% of “light” while PMMA transmits about ~40%.
  - Quartz is ideal but expensive.
- Paired or matched cuvettes are best but it is OK to use non-paired.

Color

- Color @ 430 nm, visible spectrum
- Any cuvette is acceptable for color measurement.



Errors

- Incorrect preparatory procedure
  - DE vs non-DE filtered pH
    - Problem: Not in control according to process specifications
    - How was it found: Caught in trend review
    - Correction: Updated SOP

- Incorrect sampling procedure
  - Pigtail vs no pigtail bitterness units test
    - Problem: BU was out of specification
    - How it was found: Another person performed the recheck and the error in sampling procedure was caught
    - Correction: Retrained group on correct procedure

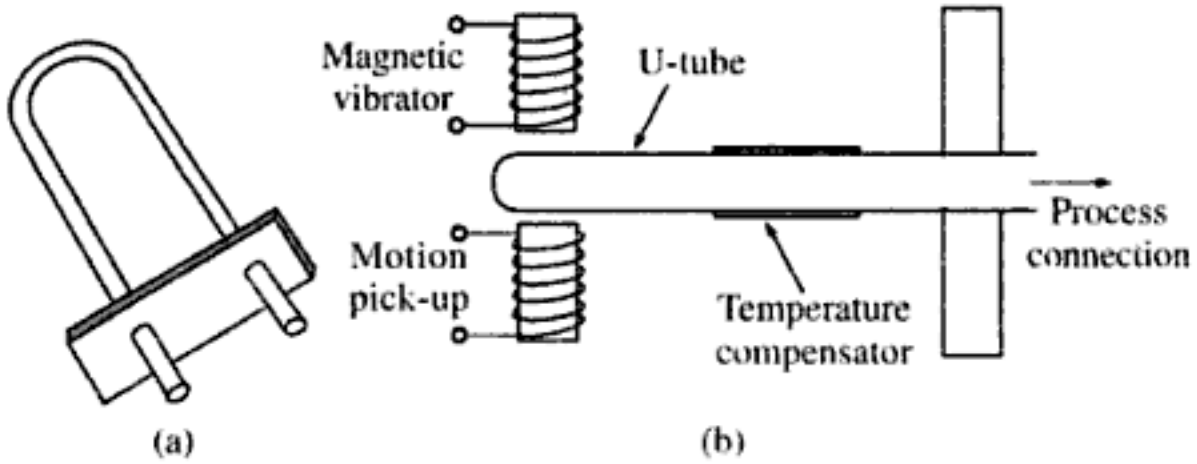
Questions?

- Thank you to:
  - Lagunitas Brewing Company
  - Rebecca Newman
  - Jessica Davis

Contact info: Aaron.Golston@Lagunitas.com

Appendix - Alcolyzer

- Densitometer
  - A U-tube densitometer is a highly accurate machine that measures the density of liquids by using an oscillating borosilicate glass U-tube.

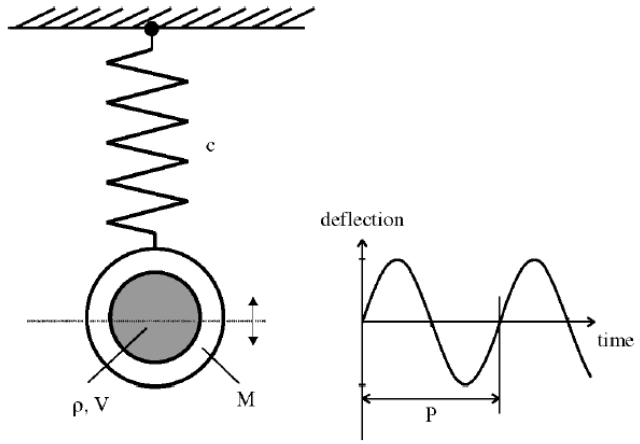


(a) The U – Tube, and (b) the general set-up

- The principle by which the unit functions is based on the simple mass on a spring concept presented in basic physics courses.
- Temperature, pressure, and flow rate play a role in the density determination but their effect is beyond the scope of this presentation.

Appendix - Alcolyzer

- The basic assumptions of the mass on a spring model are:
  1. The mass of the spring must be small compared to the attached mass.
  2. The spring must be stiff (large spring constant, c).



  - The period of oscillation, $P = 2\pi\sqrt{\frac{m}{c}}$, is dependent on the spring constant (c), which does not change and the mass of the connected U-tube, $m = M + r * V$, where M = the mass of the empty, dry U-tube, r = the density of the liquid, and V = the volume of the U-tube.
  - The only unknown in the previous two equations was the density of the liquid, r, and the equations rearrange to: $r = \frac{c}{4p^2V} * P^2 - \frac{M}{V} = A * P^2 - B$

★ A and B are constants determined by the process of **air calibrating** the unit.

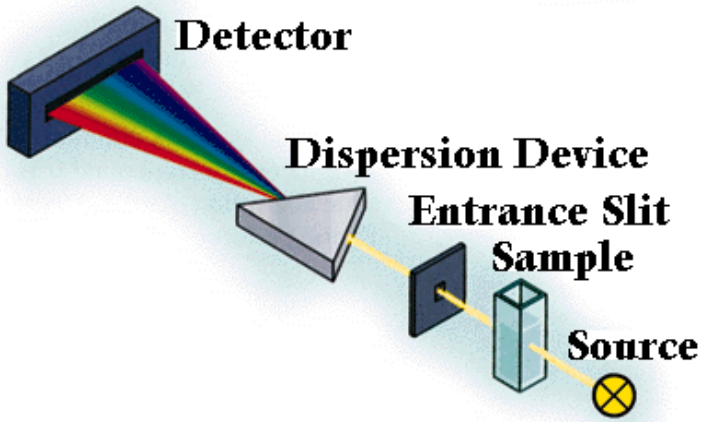
Appendix - Alcolyzer

- The densitometer measures the density of solutions in specific gravity (SG).
  - Specific gravity is measured by comparing the density of the unknown sample to that of a known reference (Distilled water).
  - Specific gravity has no units as it is a ratio but density is mass/unit volume (e.g. g/cm^3)

- Critical points to note:
  1. When air calibrating the unit it is absolutely essential that the U-tube be clean, run 1 vial of 1% sodium hydroxide followed by 4 vials of water before calibration.
  2. If the real extract (RE), determined from the SG, on your check beer is out of specification, clean the U-tube as you would before air calibrating. This should restore the U-tube to the state in which it was calibrated and everything should work correctly now.
     - If cleaning the U-tube does not work, the barometric pressure may have changed since the last calibration and the unit needs to be air calibrated.

Appendix - Alcolyzer

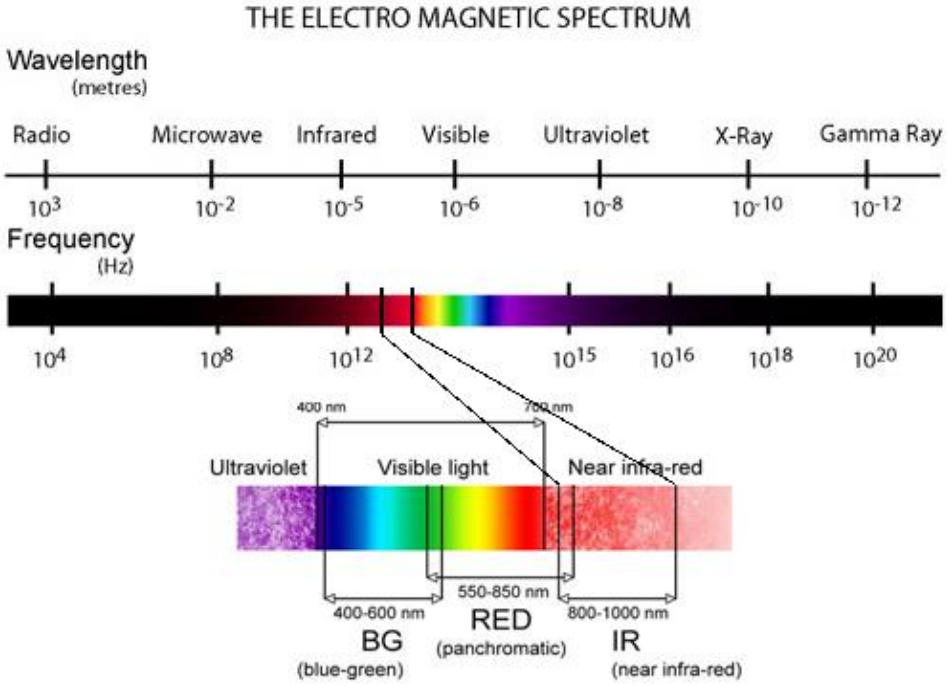
- Alcolyzer ME
  - The Alcolyzer measures the ethanol content of the sample by near-infrared (NIR) spectroscopy.



  - A light source shines through the sample and the light absorbed by the sample is determined by the detector. The amount of light absorbed by the sample is proportional to the quantity of ethanol present as calculated from the Beer-Lambert Law.
  - The Beer-Lambert Law, $A = \epsilon BC$, defines a linear relationship between the amount of light absorbed (A) and the concentration of ethanol in the sample (C). ϵ is the molar absorptivity constant for ethanol and B is the path length the light travels through the sample.

Appendix - Alcolyzer

- The electromagnetic (EM) spectrum is the range of all possible electromagnetic radiation frequencies.



- The Alcolyzer only measures in the NIR. The wavelengths measured are from 800 – 1000 nm.
- As can be seen, this is only a tiny portion of the entire EM spectrum.

Appendix - Alcolyzer

- The Alcolyzer ME system can read out ethanol measurements in many units but our preferred units are volume ethanol/volume water (v/v) and it is expressed as a percentage (%).

- The Alcolyzer is calibrated using a two-point calibration (SOP) using distilled water as a zero and a water/ethanol sample to create the other point.
  - During the calibration, the DMA 4500 tells the Alcolyzer what the alcohol content is based off of density tables contained within the unit.

  - Critical Points to note:

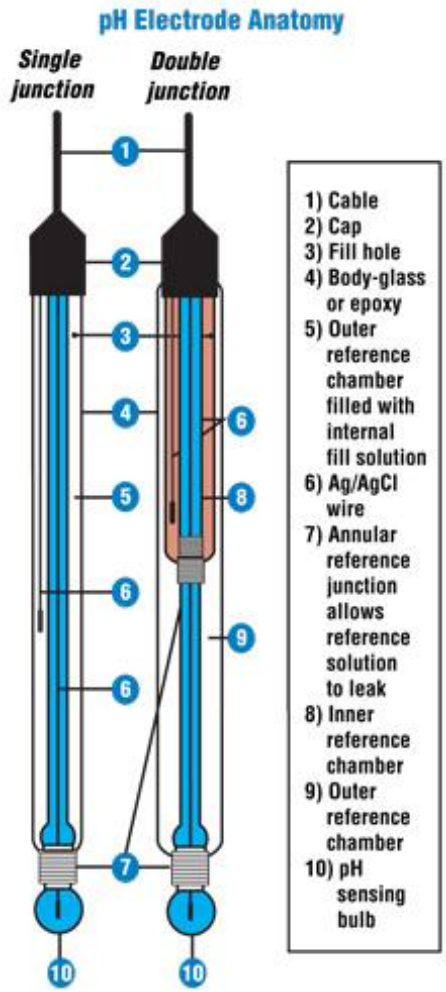
1. If the densitometer is dirty during SOP calibration, the alcohol will read off and give falsely high or low readings.
2. If the alcohol on your validation standard is out of specification, SOP calibrate the Alcolyzer.
   - If this does not fix the issue, clean the unit with 1 vial of 1% sodium hydroxide followed by 4 vials of distilled or deionized water and air calibrate the unit.

Appendix - Alcolyzer

- Alcolyzers can generate a ton of data, all of which depends on valid measurements from both the DMA and Alcolyzer.
  - Specific Gravity (DMA only)
    - $SG_{sample} = \frac{\rho_{sample}}{\rho_{water}}$
  - Apparent Extract (Plato) (DMA only)
    - Calculated from the sample using the Plato table
  - Alcohol % (v/v or w/w) (Alcolyzer AND DMA)
    - $EtOH\ \%\,{}^{w}/_{w} = \frac{alcohol\ \%\,{}^{v}/_{v} * \rho_{100\%\ EtOH}}{\rho_{sample}}$
  - Real Extract % (w/w) (Alcolyzer AND DMA)
    - Calculated by the Tabarie formula using the Plato table.
      - $SG_{real\ extract} = SG_{sample} + 1 - SG_{alcohol}$
  - Ratio (Real extract / Alcohol) (Alcolyzer AND DMA)
    - $Ratio\ {}^{E}/_{A} = \frac{Real\ Extract}{EtOH\ \%\,{}^{w}/_{w}}$
  - Calories (Alcolyzer AND DMA)
    - ${}^{kcal}/_{12\ oz} = [6.9 * EtOH\ \%\,{}^{w}/_{w} + 4 * (RE - 0.11)] * SG_{sample} * 3.55$

Appendix - pH Measurement

- There are two styles of pH probes and we use double junction probes.
- Protons pass through the bulb until an equilibrium is established with the solution being measured. Then the charge built up inside the probe is measured and the pH is determined by the Nernst equation (not shown).
- In a double junction probe, the reference electrode is separated from the outer reference chamber by junction. This slows the rate of electrode poisoning by reducing the migration of ions that can form other silver salt compounds, than AgCl, and destroy the electrode. Another junction separates the outer chamber from the solution so if this chamber fowls it protects the reference electrode.
- The chambers are filled with saturated KCl as it is equitransferent. This means as it dissociates both the potassium and chloride ions migrate across the junction at the same rate minimizing the potential for a charge imbalance to form within the electrode.

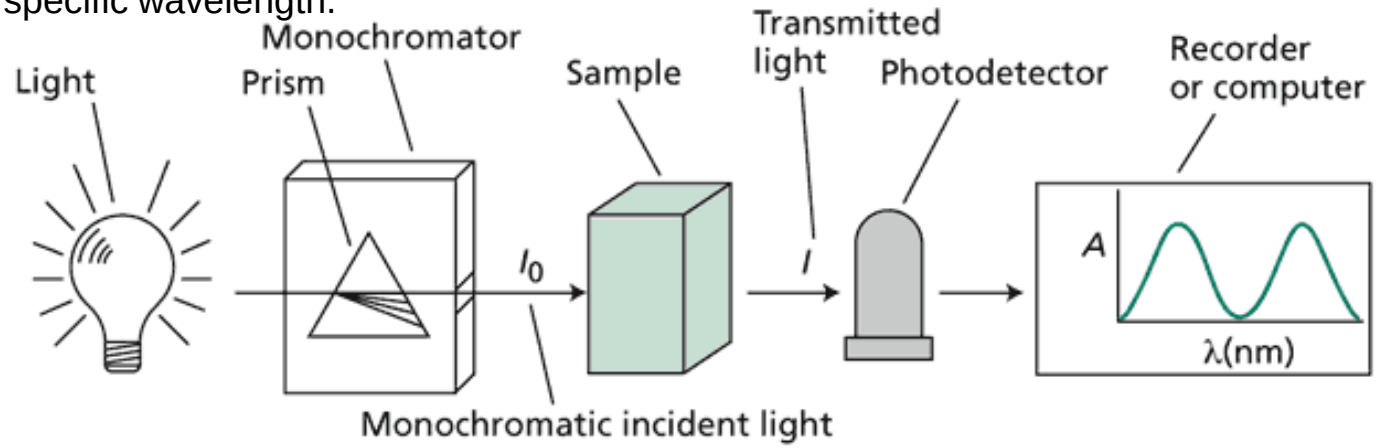


Appendix - pH Measurement

- Critical Points:
  - Make sure the reference chamber is always full of KCl solution and the air hole is open.
  - Allow solution to stabilize before accepting the value.
  - Always calibrate the pH meter for the range in which you will be measuring.
    - Beer is generally around pH 4 so the calibration range should be from pH 4 to pH 7.
    - Yeast is usually around pH 4 so the calibration range will need to be defined by the process.
  - Always store the probe wet! Clear pH 4 buffer is best.
  - Non-buffered solutions (i.e., water) will take longer to equilibrate. This can often take several minutes and is completely normal.
  - Over time the bulbs pores will clog and response will become sluggish. The electrode needs to be cleaned at this point by a 15 minute soak in 0.1N HCl. Rinse thoroughly after soak and recalibrate before use.
  - **NEVER** submerge pH probes in known solutions in excess of pH 14. These solutions can destroy a probe in a matter of hours.
  - **NEVER** measure hydrofluoric acid solutions as it will dissolve the glass at the electrode junction. (Most breweries don't have this chemical but it's just good to know.)

Appendix - Spectrophotometer

- Spectrophotometer
  - A spectrophotometer measures the amount of “light” (radiation) absorbed by a sample at a specific wavelength.



  - The wavelengths available for measurement with this device are in the ultraviolet (UV) or visible spectrums.
  - Light is shown into a prism or diffuser and then the wavelength desired for measurement is selected by moving a slit or monochromator to only allow that wavelength incident to the sample.
  - The baseline, I_0, is established before measuring the sample and the amount of transmitted light through the sample, I, is detected by the photodetector.
  - The transmittance of light, T, through the sample is expressed as a ratio: $T = \frac{I}{I_0}$

Appendix - Spectrophotometer

- Absorbance is determined from transmittance: $A = \log\frac{I_0}{I} = \log\frac{1}{T}$
- Concentration determination is done with the Beer-Lambert law: $A = \epsilon BC$
- The Beer-Lambert Law defines a linear relationship between the amount of light absorbed (A) and the concentration of the analyte in the sample (C). ϵ is the molar absorptivity constant for the analyte of choice and B is the path length the light travels through the sample.

Appendix - Spectrophotometer

- The UV spectrum spans from 100 – 380 nm and the visible spectrum spans 380 – 760 nm.