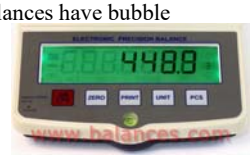
Fundamental Techniques and Measurements

- Mass Measurements
- Volume Measurements
- Preparation of a solution of known concentration
- UV-Visible Spectrophotometer

Electronic Balance

- What does an electronic balance measure? _____
- If you took an electronic balance with a capacity of 100 g to the moon what would its range be? _____

Why do good electronic balances have bubble level indicators?



Mass: Electronic Balance

- Accuracy
  - 4 to 6 significant digits
- Calibration
  - Use known mass
  - Check weekly or when balance is moved

- Sources of error
  - Balance must be calibrated and maintained in same orientation in _____ field
  - hydroscopic chemicals: dry to constant mass first (will increase in mass rapidly as they reabsorb water on the balance!)
  - When preparing a solution of a given concentration it may be difficult to get the exact mass desired_____
  - evaporation of wet samples

Electronic Balance

Model	Capacity	Resolution
DI-100	100 g	0.0001 g
DI-800	800 g	0.01 g
DI-5000	5000 g	0.1 g

- For maximum accuracy use balance with _____ resolution possible!
- Don't forget to clean the balance if you spill any chemicals!!!!!!

Volume

- Volumetric flask
  - accuracy of _____/100 mL
- Graduated cylinder
  - accuracy of _____/100 mL
- Beaker
  - accuracy of _____/100 mL
- Pipette
  - accuracy of ± _____ for 100-1000 µL
  - accuracy of ± _____ for 10-100 µl

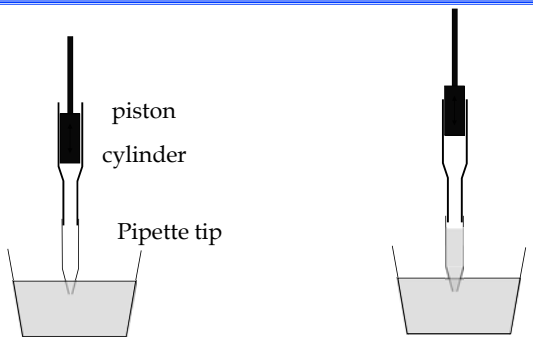
What will accuracy of solution be if you use pipette, volumetric flask, and electronic balance? _____

What controls the accuracy? __ _____

Digital Pipettes

- Air displacement
- Do not directly contact fluid volume
  - avoids contamination of pipette
  - avoids sample carryover
- Require air tight connection between tip and body

Pipette Workings



Preparation of Solutions

- Example: Prepare 100 mL of a 30 mM solution of methylene blue.
- The molecular weight of methylene blue ($C_{16}H_{18}N_3SCl$) is 319.87 g.

$$CV = M$$

$$\frac{30 \times 10^{-3} \text{ mole MB}}{\text{L}} \bullet \frac{319.87 \text{ g MB}}{\text{mole MB}} \bullet 100 \times 10^{-3} \text{ L} = 0.9596 \text{ g MB}$$

concentration ↑ conversion ↑ volume ↑ mass ↑

Preparation of Dilutions

- Prepare 100 mL of a 300 µM solution from the 30 mM solution
- Conservation of _____

$$M_{dilute} = M_{concentrate}$$

$$C_{dilute}V_{dilute} = C_{concentrate}V_{concentrate}$$

$$V_{concentrate} = \frac{C_{dilute}V_{dilute}}{C_{concentrate}}$$

$$V_{concentrate} = \frac{(300\ \mu\text{M})(100\ \text{mL})}{(30\ \text{mM})}$$

_____

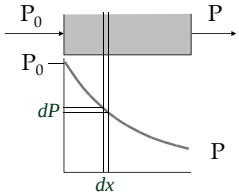
Preparation of Solutions

- Fill volumetric flask half way with distilled water
- Add reagent (could be solid or liquid)
- Mix
- Fill volumetric flask to the line
- Mix
- Verify that volume didn't change (if necessary refill to line)

UV-Visible Spectrophotometer

- Theory
- Instrument
- Sample requirements
- Software

Light Attenuation by an Aqueous Solution



P is light intensity (photons/s)

$$\frac{dP}{dx} = -kP \qquad k \propto C$$

$$\int_{P_0}^{P} \frac{dP}{p} = -\int_{0}^{x} k\,dx$$

$$\ln\left(\frac{P}{P_0}\right) = -kx$$

Theory: Light Attenuation = f(?)

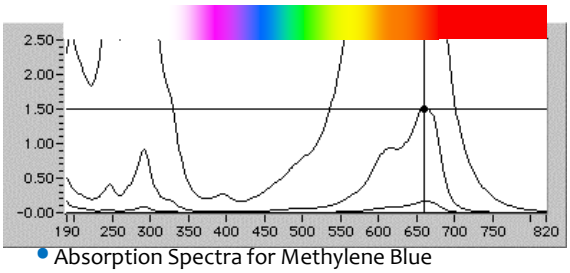
- For a given excitation process, a molecule absorbs only one discrete amount of energy: expect very narrow absorption lines.
- Different vibrational and rotational states yield _____ absorption lines.
- First order decay with distance

$$\ln\left(\frac{P}{P_0}\right) = -kx \qquad A = \log\frac{P_o}{P} = \varepsilon bc$$

$A = \varepsilon bc$

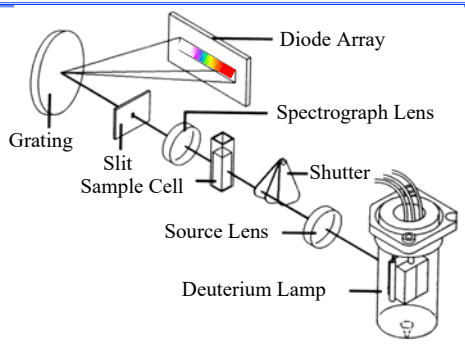
- *Po* - _____ light intensity
- *P* light intensity after passing through sample
- *b* - _____
- *c* - _____
- ε - _____ coefficient (function of wavelength and molecule)

Absorption Spectra



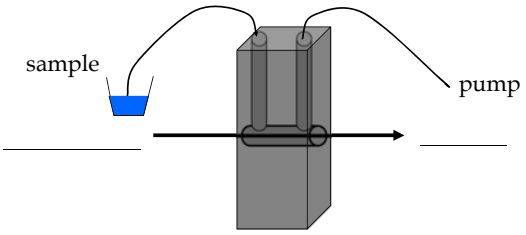
- Absorption Spectra for Methylene Blue
  - Broad peaks
  - Absorbs _____, looks _____

Instrument Light Path

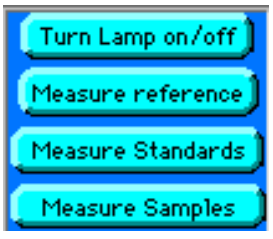


Sample Requirements

- Sipper cell
  - peristaltic pump draws sample into sipper cell
  - requires a few mL to displace previous cell contents



Software



- Reference (single sample)
  - **measures** absorbance of sample cell and reference solution
  - usually distilled water or reagent blank
- Standards (multiple samples)
  - used to create a _____ curve
- Samples (multiple samples)
  - after sampling, standards can be used to estimate the concentration of samples

Absorbance Measurement Limitations

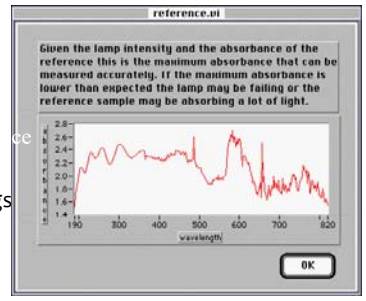
- P_o is a function of the _____.
- If absorbance is high what is P? _____
- Suppose A = 3, what is P_o/P? _____
- Suppose I create samples of higher and higher concentration. What will happen to the absorbance measurements?

$$A = \log\frac{P_o}{P} = \varepsilon bc$$

There is a _____ (non zero) P that can be measured by an instrument.
A _____ keep increasing! _____

Maximum Absorbance: P_o is measured as reference!

- Max absorbance f()
  - _____
  - _____
  - _____
  - _____
  - _____
- absorbance readings that exceed this value will not be used in analysis



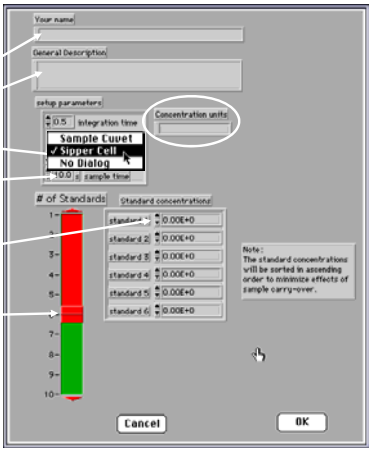
Standards

your name
general description

rinse time
sample time

sample concentrations

select number of samples by moving this control



Samples

enter sample descriptions here

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