LABORATORY MANUAL

FOR

ANALYTICAL CHEMISTRY

FIRST EDITION

DEPARTMENT OF CHEMISTRY

BABCOCK UNIVERSITY. ILISAN. REMO. OGUN STATE

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Lesson One: The Analytical Process, Terminologies and Tools of the Trade

Aims and Objectives of this study:

At the end of this experiment, the student should be able to:

- Understand what analytical processes are
- Define some common terminologies used in analytical chemistry
- Identify the common tools used in analytical chemistry

Theory

Analytical chemistry deals with identifying and determining the number and types of components (qualitative) as well as estimating the relative amount of each of these components (quantitative) in a sample. The analyte is the substance of interest in a given sample or matter. The determination of analyte in a sample gives us numerical information which is expressed in relative terms of amount of analyte per size of the sample. Application of chemical analyses can be found everywhere. Examples include determining the amount of cholesterol in a particular client, determining the reaction process (mechanism) or reaction rates, measuring the amount of caffeine in kolanut (obi, orji, goro) etc.

The analytical process involves many steps such as sampling (choosing the sample), sample preparation, extraction, separation of interfering components (filtration), sample measurement (analysis), identification, interpretation, estimation of analyte and using statistical methods to estimate error, and writing a report.

Analytical processes simply involve measurement of a specific physical characteristic (X) of an analyte that varies with its concentration C_1. The relationship between the physical characteristic and the concentration is desired to be linear: $C_1 = kX$ (k is a proportionality constant). The calculation of the value of k is important in every analytical process except in gravimetry and colorimetry and it is called Calibration constant. Desirable characteristics of a calibration standard include: Stability: (i) easily weighable, (ii) both solutions and the compound from which it is prepared must be stable (iii) Cannot be hygroscopic, deliquescent, efflorescent (iv) cannot undergo chemical change when dried, Purity(> 99.5%, Solubility, availability, Similarity with the analyte, Molecular weight should be as high as possible for easy accurate weighing, low toxicity. Few chemical compounds meet these criteria and are called primary standards

Definitions

The following are some common terms used in analytical chemistry:

- **Electrolytes**: are solutes that dissolve in a solvent to form ions that can conduct electric current.

- **Acids and Bases**: Bronsted and Lowry defined an acid as a proton donor and a base as a proton acceptor.

- **Chemical Composition**: include the components of a sample

- **Strength of Acids o Bases:** the extent of dissociation of an acid or base in a solvent will determine its strength. Some acids or base are completely dissociated in a solvent (strong acid) while others are partially dissolved (weak acid)

- **Units of Weights and Concentration:**. Mass of a substance is recorded in SI units of pg, ng, µg, mg, g, kg etc.

- **Empirical Formulas, Chemical Formula and the Mole: Empirical formula is the smallest whole number ratio of atoms in a substance.** It can be calculated from % composition of a substance. Chemical formula is the actual numbers of the atoms in a molecule. We need to know the actual molecular weight of a molecule before we can determine its chemical formula

- **The Mole**: it is a unit that expresses the amount of a substance or sample. It is associated with the chemical formula and it is equal to one Avogadro's number of atoms (6.02×10^{-23}) ions, molecules or electrons. One mole of a substance is equal to the formula weight (sum of the atomic weight of all atoms in the formula) of that substance.

- **Millimole**: The mmol is the mole divided by 1000.

- **Concentration**: the number of moles of a solute in one liter of solution or the millimoles/milliliter of solution and is called the **Molarity.**

- **Normality or Normal Concentration**: the number of equivalents of solute contained in a liter of solution. The equivalent weight is the number of moles or weight of a substance that either contributes or consumes one mole of hydrogen ions in a neutralization reaction. For strong acids or bases, the equivalent weight is equal to the molecular weight divided by the number of replaceable hydrogen ions in one mole of the acid or bases

- **Titer:** defines concentration in terms of the weight of some species with which a unit volume of the solution reacts.

- **p-Function:** sometimes, the concentration of a specie in a dilute solution is expressed in terms of its p-value. The p-value is the negative logarithm (base 10) of the molar concentration of this species: $pX = -\log[X]$.
  Density and Specific Gravity: Density is mass/volume (SI unit kg/l or g/ml) and

specific gravity is the ratio of the mass to that of an equal volume of water at 4°C (dimensionless, density of water at 4^0C =1.0).

- **Parts per Million (ppm)/Parts per billion (ppb):** It is more convenient to express concentration of very dilute solution in ppm or ppb. It is equal to amount of a solute in mg/Liter of a solution

- **Molality:** Molality, m, is the # of moles of a solute/kg of solvent.

- **Osmolarity:** total # of moles of particles/L of solution. For non electrolyte like glucose, Osmolarity is the same as the molarity. For strong electrolytes like $CaCl_2$, the Osmolarity is 3 times the molarity since $CaCl_2$ provides 3 ions in solution ($Ca + 2\ Cl^-$)

- **Percentage Composition (parts per hundred):** % composition can be expressed as follows:
  Weight % (w/w) = wt of solute/weight of solution x 100

  Volume % (v/v) = vol. of solute/vol. of solution x 100
  Weight-Volume % (w/v) = wt of solute /vol. of solution (ml) x 100

- **Solution-Diluent Volume Ratio:** The composition of a dilute solution is sometimes expressed as a ratio of the concentrated solution and the amount of solvent needed to do the dilution: For example a dilution ratio of 1:5 of HCl translates to 1 volume of concentrated HCl to 5 of the diluting solvent

- **Stochiometric Relationship:** A balance equation is needed in order to determine the combining ratio of the reactants and the products. Molar ratios of these substances give what is called the **stochiometry of the reaction**.

Tools of the Trade

The common tools employed in analytical chemistry are:

- Laboratory note Book: A laboratory notebook is used to document what was done and observed in the laboratory. The statement that "if you don't document it, then you didn't do it" is true in this situation

- Analytical Balance: Are either single pan or semi- micro balances used to weigh small size samples usually 100-200g with a sensitivity of 0.01 or 0.1 mg. This balance is a mechanical balance using built in weights to balance unknown weights.

- Electronic Balance: Unlike the mechanical balance, it does not use built in weight to return the balance to its original position. It uses the electromagnetic force to restore the balance to its original position. The major limitation of this balance is that it is calibrated

with a standard at the factory where the force of gravity is different from that of the laboratory where it is been used. Other limitations include effect of electromagnetic radiation from nearby instruments and dust getting into the gap between the coil and the permanent magnet.

- Piezo-Electric Crystal Detectors**:** measures slightest change in mass using a vibrating quartz crystal. How does it work? When pressure is applied to the crystal, voltage drop will develop across some certain surfaces of the crystal and can be used to detect so many phenomenon's such as precise time clocks.

- Burette**:** Various graduated glass cylinders are used to measure volumes of liquids. An example is the burette which is a column of glass with a graduation from 0 -50 mL. Digital Titrator: A more convenient and portable device than a burette but less accurate. It is used mostly in field operation where samples are collected. It has a dial and a counter that can be used to know how much solvent is used when collecting samples.

- Volumetric Flask: This is another type of graduated glass cylinders used in volume measurements. It is calibrated to contain a certain amount of water (50, 100 …5000 mL) at 20^{o}C. and used to prepare sample of known concentration.

- Separating Funnel: This a funnel-like glass container with different sizes of holding sections (100, 250, 500, 1000mL) and a stop-cork type of tap which is used to drain samples from the flask. It an essential tool in solvent extraction procedures

- Pipets and Syringes: Are also graduated glass columns but smaller in size compared to a burette. They are used to transfer a certain volume of solution.

- Small Volumes of Solution: Plastic micropipettes are used to deliver small volumes of solution (1-1000 µL). Accuracy is between 1-2% and precision is about 0.5%. For very small volumes, micro syringes are used instead.

- Drying: Glassware and instruments used for drying include: crucibles, evaporating dish and an oven. Different substances and wares have variable drying temperatures. The most common temperature for glassware's and crucibles is 110^{o}C. Dried substances are usually cooled down in desiccators.

Exercise

1. Define analytical chemistry

2. Akaraogun wants to measure the amount of caffeine in Kolanut. He bought 5 pieces of Kolanut from Kontagora market, cleaned the Kolanut, air dry and measured its weight. He added 20 ml of 80% methanol to the Kolanut and grinded it to a paste. The paste was

emptied into a 250 ml conical flask and 80 ml of 80% methanol was added to the paste, stirred and filtered. A brown filtrate was obtained and 1 micro liter of the filtrate was injected into the injection port of a gas chromatography/mass spectrometer for analysis (GC/MS). The GC/MS chromatogram show 8 analytes and were identified by the mass spectral in the library of the GC/MS. The external standard method (using caffeine) was used to estimate the amount of each analyte in the sample. The caffeine in the Kolanut sample was found to be 40mg/g (+/ 0.001) of Kolanut. What are the analytical processes involve in this analysis?

3. Explain the following analytical terminologies: Molar concentration, Mole, equivalent weight, Osmolarity, specific gravity, molality

4. Describe the following analytical tools: electronic balance, volumetric flask, pipette, burette, pizo-electric detector, calibration standard

5. Describe 5 requirements for a calibration standard

Lesson 2: Calibration of a Container

At the end of this experiment, the student would:

(i) Know why we need to calibrate measuring cylinders

(ii) Calibrate measuring containers

(iii) Identify measurements that requires calibration

Theory

Production of energy is very essential in building a sustainable economy. Crude oil is the most common source of energy used in all parts of the world. Crude oil is very important and it plays a very crucial part both in the economy and the politics of our present age. Nigeria is the seventh largest producer of crude oil in the world. Crude oil is extracted from the ground and transported in tanks by ships and trucks to the refinery. Along the line spillages occur and the environment becomes highly polluted. For example, the Niger-Delta region of Nigeria produces crude oil and it's highly polluted due to oil spillage. Crude oil contains mostly hydrocarbons (compounds containing carbon and hydrogen) whose densities are mostly less than 1 (density of water is 1.o g/mL) and hence it will float on water whenever there is spillage during transportation by ship or in the case of off shore drilling (which is the case in the Niger-Delta of Nigeria). Such spillages into the water ways and the environment are a great health hazard especially to the marine, inland ecosystem and the human health. It is good to determine the densities of the different hydrocarbon fraction present in the crude oil

Density =

(iv) $\frac{\text{Mass of a Liquid in grams}}{\text{Volume of Liquid in cm}^3 \text{ (mL)}}$

Apparatus: Analytical Balance, 3 liquids (G, H, I), three 10 mL measuring cylinders.

Procedure:

Calibration of a Container

Press the **ON** button to turn the balance on. Zero the balance by pressing the **Zero** button. Choose the units your desire by pressing the **Unit** button. Weigh a dry and clean 10mL measuring cylinder to the nearest 0.01g. Fill the cylinder with water and adjust the lower meniscus to exactly 10.0 mL with a pipet. Reweigh the measuring cylinder using the same balance and record your data in **Table 2a**. Calculate the volume to the cylinder to the nearest 0.1 mL by using the density of water (1.0 g/mL at room temperature). Empty the cylinder and repeat the procedure 2 more times. Find the average of the Volume of water and approximate to two significant figures. Dry the cylinder by using 2 mL of acetone. Empty the acetones into the bottle provided and allow the cylinder to dry for a few minutes.

(v) Part 2: Density of a Liquid

(vi) Label the three liquids as **GHI** and the measuring cylinder as J**, K, L** respectively. Place cylinder **J** on the balance. Allow the balance to stabilize and record the weight in **Table 2b**. Add 5.0 mL of Liquid **G** to cylinder **J** and then record the new weight. Remove cylinder **J** and repeat the same procedure for liquids **H** and **I** using cylinders **K** and **L** respectively. Return the liquids to their respective containers. Clean and dry the measuring cylinders using water and acetone. Return the measuring cylinders to their appropriate places. Complete Tables 2a and 2b and report your answer to 2 decimal places.

	Test	A	B	C	Average
1	Mass of Cylinder				Nil
2	Mass of Cylinder + 10 mL of water				Nil
3	Mass of Liquid (Line 2 - Line 1)				Nil
4	Volume of Water				
6	Density of Water	1.0	1.0	1.0	1.0

Exercise

1. Can you identify the liquids used in this experiment?
2. Calculate the density (mg/mL) of mercury if 9.75L weighs 132.5 kg
3. What is the size of 1 quart (946 mL) of corn oil if its density is 0.992 g/mL
4. An empty 25 mL cylinder weighs 54.25 g, and weighs 79.55 g when filled with water. When filled to exactly the same volume with kerosene, the weight is 75.00 g. calculate the density of the kerosene. (Density of water = 1.0 g/mL)

Lesson 3: Error Analysis I

Aims and Objectives of this experiment:

At the end of this experiment, the student should:

(i) Understand the meaning of error or uncertainty in a measurement

(ii) Be able to identify different types of error and be able to estimate them

(iii) Be familiar with different statistical approaches in estimating data and its uncertainties

(iv) Be able to apply statistical methods to analytical data

(v) Be able to determine the confidence level or interval of an analytical data

Theory

The **"true"** value of any form of measurement cannot be stated with 100% certainty. They all have a measure of uncertainty (error) which may be caused by humans, methods of analysis, instrument or from idiopathic sources. The best effort that can be made is to detect and estimate these uncertainties and try to reduce them to a tolerable level. This may include using a known reliable method, measuring the same data in different ways to see if they agree, using statistical analysis etc. The level of reliability of a data is very essential in chemical analysis and must be set by the analyst from the beginning before the data is acquired.

Errors encountered in chemical analysis can be classified into two major types: Determinate and Indeterminate errors. Determinate errors are also called **Systematic errors**. They are unidirectional, i.e. it could either be low or high and can be detected. Examples of sources of determinate error include: **instrumental, method, personal or human, and gross mistakes. Determinate errors** can be constant or proportional. Constant errors are made for every measurement made during analysis and its magnitude is independent of the number of determinations made but significant as the size of the quantity to be measured decreases. Proportional errors on the other hand, increase or decrease and are proportional to the number of replicates of the quantity to be measured. **Indeterminate errors or random error i**s due to natural limitations on our ability to make physical measurements. It could be negative or positive (random fluctuation) and it is the ultimate limitation on determining the quantity of an entity. For example, different people might read a scale differently due to different interpretations of the interpolations of the markings. Also people might give different reports of an accident they witnessed. The sources and the magnitude of these errors cannot be eliminated, corrected or quantified. The effect of this error on results is that it makes it varies randomly.

Essential Terminologies in Chemical Analysis

Reliability of a Data: Often, scientist repeats an analysis 3-5 times or more. The data obtained may not be exactly the same but will show how reliable these set of data are. Oftentimes, a "central best value" can be determined for this set of data which may be very close to the "true" value of the parameter of interest. The "central best value" may be represented by the **mean or median**.

- **Mean:** The mean, average or arithmetic mean, is one and the same expression: It is the sum of a set of replicate data/the number of individual results in the set. ($x = \Sigma x_i/N$)
- **Geometric mean:** nth square root of the product of all the results of a data: $\sqrt[n]{\prod x_i} = (\prod x_i)^{1/n}$. where $\prod$ = product of all results in the data.
- **Median:** the number at the middle of a set of data (odd set of data) or the data which have half of the set above and below it.
- **Precision:** It is the measure of the reproducibility of a data or agreement of a set of measurement obtained in an identical way. There are different ways of expressing precision. The most common way is to find the average of the deviation from the mean or median of the set of the data, $\Sigma(x_i - \bar{x})/N$. Absolute values of the deviation is used in the calculation irrespective of the sign. Other methods of expressing precision include measuring the range (spread), the standard deviation or the variance of the data.
- **The Range of a data (spread, w):** Measures the difference between the highest and lowest value in a data. Using the data in Table 2.1, the Range (w) = 5.8 – 5.1 = 0.7

Standard Deviation: This is the square root of the average of square of deviation from the mean. For N number of data, the **s** or $\boldsymbol{\sigma} = \sqrt{\Sigma(\bar{x}_i - x)^2/N}$ for large set of data and s or $\boldsymbol{\sigma} = \sqrt{\Sigma^N_{i=1}(\bar{x}_i - x)^2/(N-1)}$ for small number of data or degrees of freedom. s= the experimental standard deviation while σ = the theoretical standard deviation

- The standard deviation has the same unit as the data and is the preferred choice of expressing precision. Using the data in Table 2.1 as shown in Table 2.2

Estimation of Standard Deviation (s) from the Spread (w)

For small replicates of data ($N \leq 15$), **s** can be estimated form the spread (w)

s = w/d, where d is statistical factor that depends on the value of N

d can be approximated to $\sqrt{N}$ so $\mathbf{s = w/\sqrt{N}}$

However, this method is not as precise as using the mean deviation method

Variance: This is the square of the standard deviation (σ^2): The advantage of using variance over the standard deviation is that variance is additive i.e. if there are various causes of variance in a data, the overall variance is the sum of each individual variances of the data ($\sigma_1^2 + \sigma_2^2 + \sigma_3^2 + \ldots..$).

Accuracy: Is defined as the nearness of a data to its accepted value. It is reported as **absolute error (E). $E = x_i - x_t$.** x_i = observed data x_t = accepted value.

Relative Error (RE): Relative error is a more useful parameter than the absolute error. It is expressed as the ratio of the absolute error to the accepted value in % or parts per thousand (ppt.).

Distribution of Data from Replicate Measurements

Indeterminate errors are often small in size but cannot be eliminated from measurements. To understand how small quantities (errors) affect the outcome of replicate data, we may assume that only four errors causes indeterminate error in an experiment. The probability of these errors occurring is the same and can only affect the final result in two ways: (1) cause the outcome to be an additive error (ii) or a subtractive error of a fixed amount, U. The four errors (u_1, u_2, u_3, u_4) can combine in different ways to give different result of indeterminate errors. The ratio of the different combinations of these errors (6:4:0:1) is a probability of distribution of how the error will occur. If the number of replicate measurements is large, and the uncertainties becomes smaller and smaller, the distribution of the frequency of the probability of error will look like a bell and is called the Gaussian or normal error curve shown below

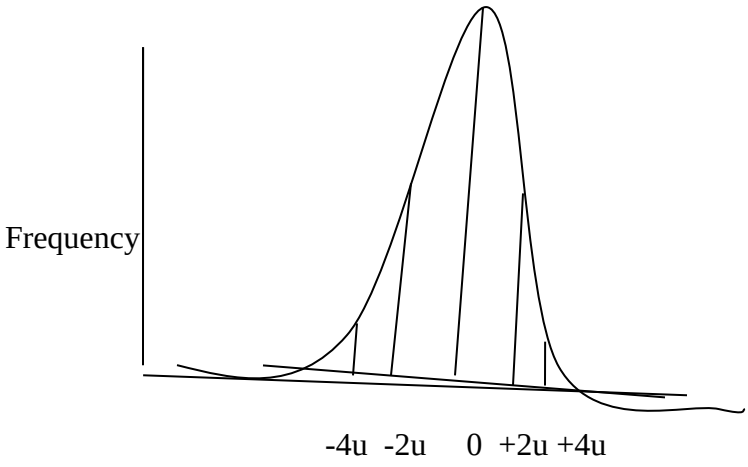


Figure 2.1 Frequency of Error vs. Indeterminate Error

Properties of the Gaussian Curve

- Has a maximum frequency of occurrence of zero indeterminate error
- It has a symmetry on both sides of the maximum indicating that there is an equal frequency of occurrence of both negative and positive errors
- There is an exponential decrease in frequency of error as the magnitude of the error increases which indicates that small indeterminate errors occur more frequently than large ones
- Indeterminate errors in chemical analysis distribute themselves in a way that approximates the Gaussian curve and it allows statistical techniques to be used in estimating the limits of precision of indeterminate errors from the precision of chemical data. Figure 2.1 can be described mathematically as follow**:** $\mathbf{y = e^{-(x-\mu)2/2\sigma2}/\sigma\sqrt{2\pi}}$ **(1)**

  where **x** = values of individual measurements, **μ =** arithmetic mean, **(x-μ)** = deviation from the mean, **y** = frequency of occurrence for each value of (x-μ), **σ =** standard deviation (a constant), **π** = 3.14 (22/7), **e** = Napierian logarithm (2.718).
- The breath of the normal error curve is related to **σ**
- If $\mathbf{z = -(x-\mu)/\sigma}$**, then equation 1 becomes** $\mathbf{y = e^{-z2/2}/\sqrt{2\pi}}$ **(2)**
- z gives the deviation from the mean a unit of σ
- **Hence plotting y vs. z will produce the same error distribution curve for all values of σ**

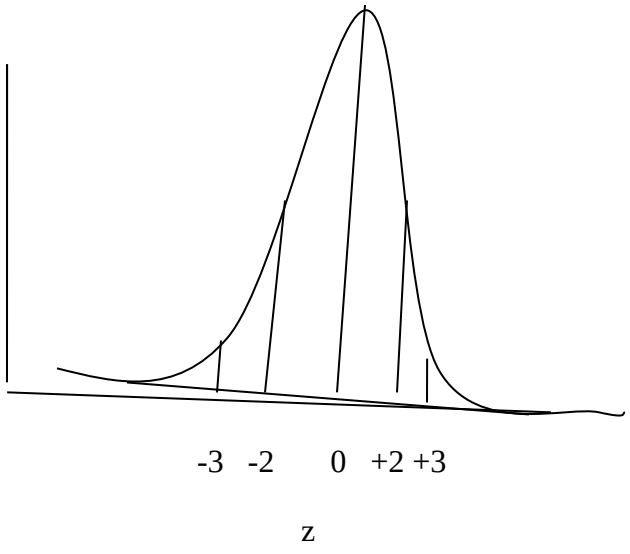


Figure 2.2 Frequency of Error vs. z

Exercise

1. Calculate the mean and median of 5.1, 5.8, 5.6, and 5.5?
2. Calculate the arithmetic and geometrical average, median, standard deviation and the variance of ten injectors on a gas chromatograph at the following speeds 40 42 45 48 50 50 55 57 58 60 m/s?
3. Determine the range and the standard deviation using the spread method of the data in question 2?
4. Estimate the precision and the accuracy of the data in exercise 2, if the accepted mean is 51.5 m/s?

Lesson 4 Error Analysis II- Application of Statistics in Chemical analysis

Aims and Objectives of this experiment:

At the end of this experiment, the student should:

(i) Be familiar with different statistical approaches in estimating data and its uncertainties

(ii) Be able to apply statistical methods to analytical data

(iii) Be able to determine the confidence level or interval of an analytical data

Theory

The Use of Statistics in Chemical Analysis

Statistical methods are used to evaluate data so that judgment about the effect of determinate and indeterminate errors can be better assessed. Some of the applications are as follow: (i) Helping to define the interval around the mean of a set of data within which the rue mean can be expected to be found with a certain amount of probability (ii) determining the number of replications needed for a set of measurement so that the experimental mean can be included with a certain probability within a predetermined interval around the true mean (iii) as a guide as to decide whether an outlying data should be rejected or accepted in calculating a mean for the set (iv) to estimate the probability that two samples analyzed by the same method are significantly different in composition, i.e. to determine that the difference in experimental result is due to indeterminate error or truly due to differences in composition or due to a determinate error (v) estimation of the probability that there is real difference in precision between two methods of analysis

The **true mean (μ)** value of a replicate of measurements is a constant that must remain unknown. Statistical theory helps us to set a limit around **experimental mean ($\overline{x}$)** within which we expect to find the **true mea**n with a certain degree of probability. These limits are called **confidence limits** and the interval of the limits is called **confidence intervals** (distance between two limits). The size of the interval depends on the degree of the correctness desired. As the probability of desired correctness increases or decreases so also the size of the interval increases or decreases. For correctness to be absolutely correct, we must choose an interval about the mean that is large enough to include possible values of the data (x_i). The interval does not have a predictive value and if we do not have to have very large interval, then we can accept a 99% level of correctness. We can even make it smaller if we choose lower percentiles of correctness.

The confidence interval obtained from standard deviation, s, for a method of measurements also depends in magnitude on the level of certainty with which it is known. Oftentimes, scientist assumes that the experimental standard deviation, s, is a good approximation of "true" standard deviation, **σ**. **σ** may have some level of uncertainty and therefore, a large interval may be needed to obtain a reasonable level of certainty

Methods of Obtaining Good Approximation of σ

Variation in the value of σ (theoretical standard deviation) may be reduced if the number of data (N) is large. In this case, value of **s** and **σ** will be the identical. This will be true for N≥ 2. It is then possible to obtain a good value of "**s**" if the method of analysis is not too long and adequate size of sample is available for replicate analysis. It is assumed that the indeterminate error is the same therefore; **s** will be a good approximation of **σ.** For time consuming methods of analysis, precision for different set of data will be pooled together to estimate **s**. The estimated **s** is superior to that of any individual set of data

Confidence Interval when s is a good approximation of σ

The breath of a normal error curve is determined by **σ**. For any given value **σ,** the area under a part of the normal error curve relative to the total area of the error curve is related to the parameter **z (z = -(x-μ)/σ)** in the equation $\mathbf{y = e^{-z2/2}/\sqrt{2\pi}}$**.** This ratio is called the **confidence level** and is reported in percentages. The ratio also measures the probability for the absolute deviation (**(x-μ) (deviation from the theoretical mean)** to be less than **zσ**.

For a single measurement, the confidence limit for the mean of the data **μ = x ± zσ**. The value of z could be positive or negative. **For N replicates of measurements**, the confidence interval decreases by √**N**, hence the confidence limit for **μ = x ± zσ/√N.** This indicates that the confidence interval can be drastically reduced as the number of measurements increases. For example, if N is 4, the confidence interval will be halved. As the value of N increases, then the law of diminishing returns sets in and there will be no more advantage of making more measurements. Therefore, it is safe to take averages of 2- 4 measurements to achieve a reasonable confidence interval for any method of analysis.

Confidence Limits when σ is Unknown (Student t-test)

When a new method is developed and used to analyze a set of data, accurate estimation of the σ may be difficult. The accurate estimation of σ may also be hampered by length of time for analysis and the amount of sample available. The same data will be used to estimate the mean and the precision which may introduce a great deal of uncertainty to the value of **s** (standard deviation). To account for the great variability of **s**, we may use the **t**-value where **t** = x-μ/**s** and it is used to measure probability, establish confidence interval, measure differences between results of the same data and also compare results from different experiments. Unlike **z**, **t** depends on the confidence level (**s**) and the degree of freedom used to calculate **s.** As the degree of freedom increases **t →z.** Values of **t** can be found in the t-table. The confidence limit can be derived from the **t-table** as μ= x ± **ts**/√**N**

Difference between Two Methods

The t-test can also be used to compare two methods of analysis. For example, methods A and B were used to analyze 6 blood samples for cholesterol. Each sample is a different blood with different cholesterol content. The results are listed in the table below. Method B gives a lower result than method A in five of the six samples. Is method B systematically different from method A?

$t = d/s_d.\sqrt{n}$ and $s_d = \sqrt{(d_i-d)^2/n-1}$

where d = average difference between the two methods and s_d = the standard deviation of the average difference of the two methods

Comparing two Means

Student's t- test can also be used to compare results of two tests using the same method to see if they are the same or different from each other. We use the formula

$t = (\bar{x}_1 - \bar{x}_2)/s. (\sqrt{n_1 n_2/ (n_1+n_2)}$ $s = \sqrt{\Sigma(x_i - \bar{x}_1)^2 + \Sigma(x_j - \bar{x}_2)^2/(n_1+n_2 -2)}$
where $\bar{x}_1$ and $\bar{x}_2$ are experimental means of the replicate data

To establish that there is a difference or no difference between the two sets of data, we must set the Null Hypothesis that μ_1 and μ_2 are identical, and if so,

$\bar{x}_1 - \bar{x}_2 = \pm ts((\sqrt{n_1+n_2/ (n_1.n_2)}$

If experimental difference is less than the calculated difference at a certain confidence level, then there is no difference between the replicate data. The converse is true.

Detection Limit

We can use the equation $x_1 - x_b = \bar{} \pm ts\bar{}((\sqrt{n_1+n_b/ (n_1.n_b)}$ to estimate the detection limit of a measurement (Δx_{min})

- $\Delta x_{min} = \bar{x}_1 - \bar{x}_b > \pm ts (\sqrt{n_1+n_b/ (n_1.n_b)}$ where
- $\bar{x}_1$, $\bar{x}_b$ and n_1, n_b are the mean and number of data for the result and the blank respectively

Rejection of Data

Sometimes we have a result that is larger or smaller (outlying) than the average of a data. We have to decide to either reject or retain the data. If we set a limit that makes rejection too difficult, we will have a false or bogus result that will have a deleterious effect on the average of the data. If we set a limit that makes rejection easy, we will discard result that should not be

discarded, hence introduce a bias to the data. The Q test is the preferred method of deciding whether to retain or reject a measurement. It is used to treat individual data

Q = (Questionable result – Nearest result to Questionable result)/Range of result (spread)

If Questionable result – Nearest result to Questionable result) = Gap, then

Q = Gap/Rang. There is a table of **Q** values at different confidence level which can be used to calculate the values of Q for any data

If $\mathbf{Q_{(observed)} > Q_{(tabulated)}}$, then, the data should be rejected but if $\mathbf{Q_{(observed)} < Q_{(tabulated)}}$, then the data should be retained

The **Q** test is not that helpful for small sets of data (N < 5) because if a result has less than 10% of being real and is included in a data, it will introduce serious bias into the data. It is better to reexamine existing data to spot sources of error or spend time to repeat the test if more sample is available than out rightly retaining or rejecting the result. However, If the Q test suggest retaining the result, then report the median rather than the mean because the median has a higher virtue of including all data than the mean

Comparison of Precision of Measurements (F-test)

The F-test is used to compare the precision of two identical sets of data. The sample can be the same sample or different samples that are sufficiently similar so that the sources of indeterminate error are the same. F –test is based on the null hypothesis that the precision of the two sets of data are identical. The F-value is the **ratio of the variances of the two sets of data.** The experimental F_{exp} value is compared to the maximum F_{tab} value obtained at a certain confidence level from the tables of F values (at specific degrees of freedom) if there are no differences in precisions

$F_{exp} > F_{tab}$, then there is a statistical basis for questioning whether the two standard deviations are alike. The **F**- value can be used to answer two pertinent questions: (1) if $method_1$ is more precise than $method_2$. In this case, the standard deviation of the more precise method is made the denominator and the less precise one the numerator (2) If there is a difference in the precisions of the two methods. In this case, the larger variance is made the numerator. Making the larger of the variances the numerator increased the outcome of the test **less certain,** hence the level of confidence may be decreased

Exercise

1. The mercury in seven samples of fish taken from Esinmirin River was determined by a method based upon absorption of radiation by elemental mercury. The results are given in the table shown below. Calculate a standard deviation for the method,

Sample #	# of Replicates	Results, Hg ppm
1	3	1.80,1.54, 1.64
2	4	0.96, 0.98, 1.02, 1.10
3	2	2.13, 3.35
4	6	2.06, 1.93, 2.12, 2.16,1.89,1.95
5	4	0.57, 0.58, 0.54,0.49,
6	5	2.35, 2.44, 2.70, 2.48, 2.44
7	4	1.11, 1.15, 1.22, 1.04

2. A chemist obtained the following data for the alcohol content in a sample of blood: percent of alcohol; 0.0840.089 and 0.079. Calculate the 95% confidence limit for the mean assuming (a) no additional knowledge about the precision of the method and (b) that on the basis of previous experience, s→ σ 0.006% ethanol.

3. The composition of a flake of paint found on the clothes of a h it-and run victim was compared with that of the paint from the car suspected of causing the accident. Do the following data for the spectroscopic analysis for titanium n the paints suggest a difference in composition between the two materials? Form previous experience, the standard deviation for the analysis is known to be 0.35% Ti; that is, s→ σ Paint from clothes %Ti = 4.0, 4.6. Paint from car %Ti = 4.5, 5.3, 5.5, 5.0, 4.9

4a. A method for the analysis of DDT gave the following results when applied to pesticide –free foliage samples (µg): 0.2, -0.5, -0.2, 1.0, 0.8-0.6, 0.4, 1.2. Calculate the DDT detection limit (99% confidence level) for (a) a single analysis and (b) the mean of five analyses

4b. The amount of chloride ion in a factory waste was determined by titrimetric method. Five replicates were obtained: 12.53, 12.56, 12.47, 12.67 and 12.48. Is 12.67 a bad result?

5. The standard method for the determination of carbon monoxide in gasesous mixtures is known from many hundreds of measurements to have a standard deviation of 0.21 ppm CO. A modification of the method has yielded an s of 0.15 ppm CO for a pooled data with 12 degrees of freedom. A second modification, also based on 12 degrees of freedom, has a standard deviation of 0.12 ppm Co. Is either of the modifications significantly more precise than the original?

Lesson 5: Error Analysis III -Propagation of Error

Aims and Objectives of this chapter:

At the end of this chapter, the student should be able to

(i) Propagate determinate errors in processes that include: addition, subtraction, multiplication, and division

(ii) Propagate indeterminate errors in processes that include: : addition, subtraction, multiplication, division, logarithms and antilogarithm

(iii) To draw calibration curves using the method of least squares

(iv) Estimate errors in least square methods

(v) Use calibration curves to estimate the amount of analyte's in a sample with its errors

Theory

Propagation of Error

Any result obtained from every experiment has a form of error associated with it. The error could be a determinate or indeterminate error and both have different effects on the result. In some cases, the error will cancel out and in other cases, it will not and accumulates to a significant proportion. These errors may be accumulated in different ways depending on the arithmetic relationship between the term containing the error and the item that is being measured. Errors or uncertainty in results is expressed as standard deviations of replicate determinations.

Accumulation of Determinate Errors

Method of accumulation of errors in addition/subtraction is different from that of multiplication and division

Addition/Subtraction

If a measurement is represented by the equation $y= a+ b-c$ (1)

where a, b, c are values of three different measurements. Each measurement will have absolute determinates errors Δa, Δa, Δb associated with them respectively.

So, the actual result obtained for each of a, b and c is $y + \Delta y = (a+\Delta a) + (b+ \Delta b) – (c + \Delta c)$ (2)

Subtracting equation (1) from (2) will give the absolute error Δy of the overall measurement as

$$\Delta y = \Delta a + \Delta b – \Delta c$$

This implies that the overall error of a data involving addition or subtraction is determined by the sum/difference of errors associated with the individual measurements that makes up that data

Multiplication

Consider the product $y = a \times b$ (1)

with associated absolute errors $\Delta a + \Delta b$ and overall error of Δy

: $y + \Delta y = (a+\Delta a)(b+ \Delta b) = ab + a\Delta b + b\Delta a + \Delta a\Delta b$ (2)

Subtracting (1) from (2) $\Delta y = b\Delta a + a\Delta b + \Delta a\Delta b$ (3)

If we divide equation (3) by equation (1):) $\Delta y/y = \Delta a/a + \Delta b/b + \Delta a\Delta b/ab$

$\Delta a\Delta b/ab$ will be a small term compared to $\Delta a/a$ and $\Delta b/b$ because of the larger denominator and the product of two small numbers, hence $\Delta y/y \sim \Delta a/a + \Delta b/b$

This error is a relative error not absolute errors as in the case of addition/subtraction

Division

Consider the product $y = a /b$ (1a) or $yb = a$ (1b)

with associated absolute errors $\Delta a + \Delta b$ and overall error of Δy

: $y + \Delta y = (a+\Delta a)/(b+ \Delta b)$ (2a) or

$(a+\Delta a) = yb + y\Delta b + b\Delta y + \Delta y\Delta b$ (2b). substituting eqn (1b) in (2b),

$(a+\Delta a) = a + y\Delta b + b\Delta y + \Delta y\Delta b$ (3a) and

$\Delta a = y\Delta b + b\Delta y + \Delta y\Delta b$, dividing by eqn (1)

$\Delta a/a = y\Delta b/yb + b\Delta y/yb + \Delta y\Delta b/yb$ (4a)

$= \Delta b/b + \Delta y/y + \Delta y\Delta b/yb$

$\Delta y/y = \Delta a/a- \Delta b/b-\Delta y\Delta b/yb$ assuming that $\Delta y\Delta b/yb <<<<< \Delta a/a- \Delta b/b$, then

$\Delta y/y = \Delta a/a- \Delta b/b$

In general, if $y = ab/c$ then $\Delta y/y = \Delta a/a+ \Delta b/b - \Delta c/c$

Accumulation of Indeterminate Errors

Standard deviation is the most convenient way of representing indeterminate errors however; no particular sign can be attached to these standard deviations when it comes to indeterminate

errors. Each of these errors have equal chances of been positive or negative, hence we attach the sign ± to standard deviations of indeterminate errors.

Addition/Subtraction

The most probable error in summation or differences of indeterminate errors in a given data is given by the square root of the individual variances of each of the measurements that constitute that data: $s_y = \sqrt{s_a^2 + s_b^2 + s_c^2}$

Multiplication and Division

Just like we did with determinate errors, we must first of all calculate the relative errors for each measurement ($\Delta a/a$, $\Delta b/b$, $\Delta c/c$) and then relative variance of the result ($s_y = \sqrt{s_a^2 + s_b^2 + s_c^2}$) and finally find the absolute error of the result ($\Delta y = s_y \times y$)

Mixed Operation

1. $[14.3(\pm0.2) - 11.6(\pm0.2)] \times 0.050(\pm0.001)/[820(\pm10) + 1030(\pm5)] \times 42.3(\pm0.4) = 1.725\ (\pm?) \times 10^{-6}$
2. $[1.763(\pm0.03) - 0.59(\pm0.02)]/1.89(\pm0.02) = 0.691\ (\pm?)$

Propagation of Errors in Exponential Determinations

Consider an experimental result, **a**, expressed as

$y = a^x$, (1), x is the power or root that contains no uncertainty.

The uncertainty Δy due to determinate error Δa in **a**, is determined by taking the derivative of y wrt **a,**

$dy = xa^{(x-1)}da$ (2)

Divide eqn 2 by eqn (1)

$dy/y = xa^{(x-1)}da/a^x$ (3) but $a^{(x-1)} = 1/a$ hence eqn(3) becomes

$dy/y = xda/a$

For finite increments

$\Delta y/y = x\ \Delta a/a$ where Δy is the absolute error of the data while $\Delta y/y$ is the relative error of the data. This implies that the relative error is the product of the power or root (exponent) of the data and the relative error of the data itself. So if x= 2, the relative error is double of the error of the data and if x = ¼, the relative error will be ¼ of the error of the data itself

This equation applies to indeterminate errors itself where s_y = x s_y

Propagation of Error in Logarithm and Antilogarithm Calculations

If y = log **a**, then the natural logarithm of log **a** = In **a**

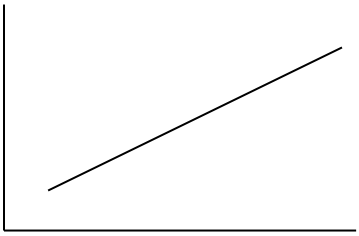
So, y= loga = 0.434In **a**. Taking the derivative of y = dy = 0.434da/**a**

For finite values $\Delta y = 0.434\ \Delta a/\mathbf{a}$. this implies that the absolute uncertainty in y is equal to the relative uncertainty in **a**.

The absolute standard deviation = $s_v = 0.434\ (s_a)_r$

Calibration Curve

Nearly all analytical method needs a calibration curve. The standard and the sample are treated exactly in the same way. Different concentrations of the chosen standard sample are used to obtain data and the data is plotted against the concentrations to obtain a calibration curve as shown below.



A region of the concentration where all the data points will fit a straight line is the most desired. This region will give a one: one (1:1) correspondence between the size of the desired variable and the concentration of the standard and is then used to estimate the size of an analyte in a sample. Usually, not all data points will fall on a straight line because of indeterminate error. Hence, the scientist need to find the **best straight line** that fits the data. Regression analyses allow such a line to be obtained and also furnish the uncertainty associated with the line. The regression method is called **method of least squares.**

The **method of least squares** assumes that: (i) there is a linear relationship between the analyte concentration (**independent variable)** and the measured variable (**dependent variable)** (ii) the exact concentration of the standard is known. Therefore, any deviation from such a line is due to indeterminate error in the measured variable and are similar (iii) the error in the measured variable is greater than the one in the independent variable. The linear relationship is represented by the equation:

y = mx + c [m = slope of the line and c = interception i.e. where the value of y = 0]

The deviations from the straight lines, **residuals**, [**Q= y_i- (mx_i + c)= vertical distance between the line and each data point],** are random and could be positive or negative. The **method of least squares**' aim is to minimize the sizes of the deviations and therefore, we can find the best straight line of which the squares' of the deviation (so that we only deal with positive numbers) is the minimum.

Determining the Slope, Intercept and the Standard Deviations (slope, intercept, result)

The slope, intercept and their standard deviations can be derived from the least square method by applying statistical equations. Let us define the following parameters

$A^2 = (\Sigma(x_i-\bar{x})^2 = \Sigma x_i^2 - (\Sigma(x_i)^2/n$, $B^2 = (\Sigma(y_i-y)^2 = \Sigma y_i^2 - (\Sigma(y_i)^2/n$,

$C^2 = (\Sigma(x_i-\bar{\bar{x}})(y_i-\bar{y}) = \Sigma x_i\, y_i - (\Sigma(x_i)\Sigma y_i)/n$

x_i an y_i are pairs of data, $\bar{x_i} = \Sigma x_i/n$ and $y_i = \Sigma y_i/n$ are the mean of the data pairs, n is the number of data pairs that defines the of the calibration curve. Then

$m = AB/A^2$, $c = \bar{y} - b\bar{x}$, s_r = standard deviation of the residuals = $\sqrt{B^2 - b^2A^2/n-2}$

s_m = standard deviation of the slope = $\sqrt{s^2_r/A^2} = s_r/A$,

s_c = standard deviation of the results based on this calibration curve

$= s_r/b\sqrt{1/m + 1/n + (\bar{y}_c - \bar{y})/b^2A^2}$, $\bar{y}_c$ = mean of the result for the sample, $\bar{y}$ = mean of the standard solution

Exercise

1. Calculate the error in the result of the following determinations of Cl^- ion in sea water: + 0.41 (+0.01), +0.38 (+0.02), - 0.45 (-0.04) ppm?
2. Calculate the error in the result of the following determinations of Cl^- ion in sea water: 0.41 (+0.01) x 0.38 (+0.02)/0.45 (-0.04) ppm?
3. Calculate the error in the sum of the result of the following determinations of Cl^- ion in sea water: 0.41 (±0.01), 0.38 (±0.02), -0.45 (±0.04) ppm?
4. Calculate the error of the result for Cl^- ion from sea water: 0.41 (±0.01) x 0.38 (±0.02)/0.45 (±0.04) ppm?
5. Calculate the standard deviation of the following data:
   (a) [14.3(±0.01) -11.6(±0.02)] x 0.050(±0.001)/[820(±10) + 1030(±5)] x 42.3(±0.4) = 1.725 (±?) x 10^{-6} (b) [1.763(±0.03) - 0.59(±0.02)]/1.89(±0.02) = 0.691 (±?)

6. The standard deviation in measuring the diameter, d, of a sphere is ±0.02 cm. what is the standard deviation in its calculated volume V if d = 2.15 cm?

7. The solubility product K_{sp}, for a silver salt AgX, is $4.0(\pm 0.02) \times 10^{-8}$. What is the uncertainty associated with the calculated solubility of AgX in water?

8. Calculate the absolute standard deviation of the following results:
(a) log $[2.0(\pm 0.02) \times 10^{-3}] = -0.269 \pm ?$ (b) a = antilog$[1.200(\pm 0.003) = 15.849 \pm ?$ (c) a = antilog$[45.4(\pm 0.3) = 2.5119 \times 10^{45} \pm ?$

9.

Lesson 6: Gravimetric Analysis

The aims and objectives of this lesson are:

(i) To teach the student the theory behind gravimetric analysis

(ii) To teach student how to formulate and use the gravimetric factors

(iii) To teach the student the different methods employed in gravimetric analysis

At the end of this lesson, the student should:

(i) Know that gravimetric analysis uses weight measurements to determine the amount of a substance from a sample

(ii) Be able to determine the gravimetric factor needed in determine the amount of a product in a sample

(iii) Understand that different methods such as precipitation and volatilization can be employed in gravimetric determinations.

(iv) Know the advantages and disadvantages of gravimetric analysis.

Theory

Gravimetric analyses involve using the weights of a product to determine that of the sample. It is also called **quantitative analysis by weight**. The composition of the product and sample are known and are also chemically related to each other. Gravimetric processes involve isolating the desired analyte and manipulate it to get it in its most stable purest form and in a format that can be weighed. The weight of the analyte is then calculated from chemical formula and the atomic weights of the elements that make up the analyte.

There are many methods used in gravimetric analysis: (1) **precipitation methods**: Here, the analyte to be determined is made to react with other reagents that produces an insoluble product, which can be filtered and treated to give a stable desired product of known composition. The product is then weighed, manipulated and reported in the desired format (2) **volatilization methods**: this is applicable to a volatile analyte. The sample is heated up to volatize the analyte. The weight of the analyte or left over sample (nonvolatile part) may be used to estimate the desired data (3) **electro-analytical methods**, (4) **extraction methods**, and (5) **chromatographic methods**.

The disadvantage of gravimetric analysis is that it is time consuming and the advantage is that it can be isolated and the number and amount of impurities present in the analyte can be determined.

Mass measurement is the most important aspect of gravimetry and it can be done with high degree of accuracy. Therefore, gravimetric method is commonly used in analytical chemistry. The only instrument used in gravimetry is a balance. Most precipitating agents are non specific; hence, reaction conditions must be adequately controlled to avoid co-precipitation of interfering ions. Tables of precipitating anions, cation are organic reagents are available in the literature.

In gravimetric calculations, the general approach is to compare moles of product (analyte) to that of the sample. Sometimes, the analyte sought is the product or a part of the product that is weighed, or is chemically related to the product. In all cases, the weight of the product must be converted to that of the analyte sought by a gravimetric factor. For example if the amount of Fe in $Fe_2(SO_4)_3$ is to be determined by precipitation as:

$$BaSO_4: Fe_2(SO_4)_3 + 3Ba^{2+} \rightarrow 2Fe^{3+} + 3BaSO_4.$$

The Gravimetric factor: 2x fw. Fe/3 fw. $BaSO_4$ (fw = formula weight)

In this experiment, Calcium (Ca) will be precipitated as calcium oxalate (CaC_2O_4) and dried with the oven and the furnace

Apparatus: 1x 250 conical flask, 1x 250 beakers, stirrer, weighing balance, oven, furnace, filter paper, heating mantle or water bath, 4x 10mm test tubes, centrifuge

Reagents: 0.1M Hydrochloric acid, 0.5 M ammonium oxalate, 0.05M ammonium oxalate, 0.05M ammonium hydroxide solution, calcium chloride

Procedure

Weigh between 0.2-0.4g of calcium chloride and dissolve in 25.0 mL of hydrochloric acid (HCl) in a 250.0 mL conical flask. Gently heat the HCl (do not heat to boil) for about 2 minutes. Remove solution from the heating source. Put the hot solution in the fume hood and gradually add 25.0 mL of 0.5M ammonium oxalate along the side of the flask while stirring. Neutralize the mixture with 0.05M ammonium hydroxide solution until crystals or a white suspension of the calcium oxalate is formed. If crystals are formed, filter the precipitate and wash with 20 mL of 0.01M ammonium oxalate solution. Split the sample into two equal parts. The white precipitate is calcium oxalate monohydrate ($CaC_2O_4. H_2O_s$). Use oven to dry one half of the precipitate for 30 minutes to one hour at 101-105^oC. The product is calcium oxalate monohydrate because calcium oxalate is hygroscopic at this low temperature and also ammonium oxalate co-precipitates with calcium oxalate. Cool dry samples in desiccators for 10 minutes and weigh the sample.

$$CaCl_2s + C_2O_4^{2-}{}_{(aq)} + H_2O_l \xrightarrow{\Delta} CaC_2O_4. H_2O_s \rightarrow CaC_2O_{4s}. H_2O_s$$

Dry the other half in the furnace between 475-525^oC for 15 minutes. Cool dry samples in desiccators for 10 minutes and weigh the sample. The product is calcium carbonate ($CaCO_{3s}$)

$$\xrightarrow{\Delta}$$

$CaCl_2s + C_2O_4^{2-}{}_{(aq)} + H_2O_l \rightarrow CaC_2O_4.H_2O_s \rightarrow CaCO_{3s} + CO_g$

If a white suspension is formed instead of white crystals, put the suspension in about four pre-weighed test tubes and centrifuge for about one minute. Decant the supernant solution and split the sample into two equal parts. Use the same method of drying as indicated above.

Calculate the percentage of calcium recovered from these analyses? Calculate the percentage difference in the amount of calcium obtained by the two system of drying?

Exercise

1. Why is the product of drying of the oxalate different at low and high temperatures?.
2. Weight of calcium obtained with oven drying is higher than the one obtained with furnace drying. Why?
3. A method for measurement of soluble organic carbon in sea water involves oxidation of the organic material to CO_2 with $K_2S_2O_8$ followed by gravimetric determination of CO_2 trapped by a column of NaOH coated asbestos. A water sample weighing 6.234 g produced 2.378 mg of CO_2. Calculate the ppm carbon in the seawater.

Lesson 7: Titrimetric Analysis

Aims and Objectives of this experiment:

At the end of this experiment, the student should:

(i) Understand the meaning of error or uncertainty in a measurement

(ii) Be able to identify different types of error and be able to estimate them

(iii) Be familiar with different statistical approaches in estimating data and its uncertainties

(iv) Be able to apply statistical methods to analytical data

(v) Be able to determine the confidence level or interval of an analytical data

Theory

Apparatus:

Reagents:

Procedure

Exercise

Lesson 8: Chromatographic Separation Methods: Thin Layer/Paper Chromatography

Aims and Objectives of this experiment:

At the end of this experiment, the student

(i) Understand the principle of thin layer chromatography

(ii) Would have used thin layer chromatography to separate mixtures

(iii) Would have learn about how analyte's are portioned between two phases to aid separation

(iv) would have acquired the skill of using thin layer chromatographic technique to separate mixtures

Theory

Chlorophyll is extracted from some bacteria, algae and vegetables like 'ugu'. Separation of the extract is done with a silica gel TLC plates (microscope slide sized) instead of a filter paper.

The extraction involves grinding up the 'ugu' leaf in a little bit of acetone (we want to get as much of the chlorophylls, carotenes, xanthophylls and other components out of the leaves as we can). Acetone will dissolve almost anything, including the components that are not needed. Chlorophylls, carotenes and other organic based components do not dissolve very well in water, but dissolve well in hexane. Some other compounds do not dissolve well in hexane, but dissolve well in water. Water and hexane 'do not mix.' If you mix them together, the hexane will form a layer on top of the water (like oil does) because hexane is less dense than water. After vigorous shaking the lovely green chlorophylls and other organic components will leave water and it's components at the bottom to dissolve in the hexane layer at the top. After pipetting off the hexane layer, isolating the chlorophylls, carotenes and others will be done by thin layer chromatography using a silica gel plate.

Apparatus: fresh ugu leaves, acetone, 5" Pasteur pipette, 1-15mL centrifuge tube with a screw cap OR a test tube with a tight-fitting cork, distilled water, hexane, small beaker for waste, boiling chip or stick, anhydrous Na_2SO_4, sample vial, a pencil, a ruler, cotton OR glass wool, silica gel TLC plates-microscope slide sized, filter paper, TLC chamber, UV lamp

Procedure

1. Weigh out approximately 0.5 g of fresh spinach leaves (don't use stems) and record the mass. Tear the leaves into confetti-sized pieces and place them into a mortar. Add about 1.0 mL of acetone and grind the leaves with a pestle until the acetone turns a bright, deep green. You may add more acetone as necessary.

2. Using a 5" Pasteur pipet, transfer the liquid to a centrifuge tube. Don't be too concerned if a small amount of sludge-like gunk is also transferred, it will be extracted later. Rinse your mortar and pestle with another 1.0 mL of acetone and transfer this to the centrifuge tube also.

3. If you have more than 1.5 mL of acetone in the centrifuge tube at this point, it's a good idea to reduce the volume via evaporation in a warm sand bath. Don't forget the boiling chip or boiling stick or it will spatter. Allow the extract to cool to room temperature before the next step.

4. Add 2.0 mL of hexane and 2.0 mL of distilled water to the extract in the centrifuge tube, cork it and shake gently. Occasionally, ***direct the tube away from yourself and others***, and vent. Shortly, there should be a cloudy, light green lower water layer and a clear, bright green top hexane layer. Be sure to identify which is the water and which is the hexane layer. Allow the tube to stand a few minutes undisturbed to maximize separation of these layers.

5. Using a 5" Pasteur pipet, carefully draw off the water layer and transfer it to a small waste beaker. Remember to depress your pipet bulb before you insert the pipet into the centrifuge tube.
Experiment 9 Cont'd

6. Add another 2.0 mL of distilled water to the hexane layer in the centrifuge tube as a wash. The new water layer should be very nearly colorless even after shaking and allowing the layers to separate. Again, draw off the bottom water layer and combine it with the first one in the waste beaker.

7. Although water and hexane 'do not mix'…reality is that a little bit of the water will stay in the hexane. You can tell there is water in the hexane layer; it may be a little cloudy. You must 'dry' the hexane layer by adding some anhydrous Na_2SO_4. A few micro-spatula scoops is usually sufficient. Na_2SO_4 soaks up the water as it becomes hydrated. However, you can be sure you've added enough when additional Na_2SO_4 no longer clumps when swirled in the extract and your extract is not at all cloudy. Ask your professor for help with this.

8. To remove the drying agent and filter your extract: prepare a filter paper by inserting a small cotton plug into a 5" Pasteur pipet. Using a micro-clamp, secure the pipet on a ring stand and place a clean collection vial below the stem of the pipet. Transfer your extract via pipet to the filter pipet. The cotton plug will prevent any drying agent from contaminating your extract as it drips into the vial. To complete the transfer, rinse the centrifuge tube containing the drying agent with another 1.0 mL of hexane and filter this also.

Thin Layer Chromatography

1. Once you have your hexane extract containing the extract, run TLC, (thin layer chromatography). Put a little spot of your extract on a glass or plastic plate coated with silica gel. Silica gel is a very polar substance. Then, you will stand the plate in a container with a mixture of solvents. In this case, the solvents will be quite non-polar (hexane). The solvents will begin to travel up the plate, like a wick. Some of the compounds in your mixture will be more polar and will stick to the spot on the silica. Other compounds in your mixture will be less-polar to differing degrees and will travel up with the solvent. Since there are many levels between totally polar and totally non-polar, the compounds can be separated by polarity. The more affinity a compound has for the solvent, the farther up the plate it will travel.

2. Obtain a TLC plate and ***very*** lightly draw a pencil line (no ink!) about 1 cm from the bottom. If you press too hard, the silica will come off…in which case you will have to get a new plate! Using a capillary tube, make a spot of your extract on the pencil line. You may have to let the spot dry and then spot it again if it isn't dark enough. Carefully place the spotted plate into the chamber and replace the lid. (If you drop the spot into the solvent, you will have to spot a new plate.)

1. You will immediately see the solvent start to travel up the plate. The line of solvent moving up is called the 'solvent front.' Once the solvent front is within 1 cm of the top of the plate, remove the plate and quickly mark the solvent front with a pencil.

Although most of the spots are easily visualized by the naked eye, **use the UV lamp** to insure that you are noting all possible spots. Circle each spot in pencil. Lightly label each spot (A, B, C,…).

5. Determine Rf values for all of your spots. This will give you quantitative values for comparison. Measure the distance from the starting line to the solvent front. Then, measure to the center of each spot. Divide the center spot distance by the solvent front distance; this is the Rf value of the compound.

6. Try to match them to the compounds shown below (listed in order of decreasing Rf values):

carotenes (1-2 yellow-orange spots)
pheophytin A (gray, intense)
pheophytin B (gray, may only be visible under UV)
chlorophyll A (blue-green, intense)
chlorophyll B (green)
xanthophylls (as many as 3 yellow spots)

Experiment 8 Cont'd

Experiment 8 **RESULT**

Name **Department** **Date**

Table 8-1

Compound	Color of Spot Under UV Lamp	Distance from origin to solvent front	Distance from Origin to center of Spot	R_f
Carotenes				
Pheophytin A				
Pheophytin B				
Chlorophyll A				
Chlorophyll B				
Xanthophylls				

Question

1. How many types of chlorophyll molecule exist in nature. Name them and their sources
2. Is the mechanism in separating compounds using paper or glass or plastic coated with silica slides chromatography different?

Reference Readings

1. Instrumental Analysis by Skoog and West. 3rd Edition. USA
2. Instrumental Analysis Manual. Delaware University Newark Delaware. USA
3. Quantitative Chemical Analysis by Harris Daniel