

Chapter 6

Elemental Analysis and Biological Characterization

6.1 Introduction

Percent Composition is a process where a sample of some material (e.g., soil, waste or drinking water, bodily fluids, minerals, chemical compounds) is analyzed for its elemental and sometimes isotopic composition[1,2]. Elemental analysis can be qualitative (determining what elements are present), and it can be quantitative (determining how much of each are present). Elemental analysis falls within the ambit of analytical chemistry, the set of instruments involved in deciphering the chemical nature of our world.

For organic chemists, elemental analysis or "EA" almost always refers to CHNX analysis—the determination of the mass fractions of carbon, hydrogen, nitrogen, and heteroatoms (X) (halogens, sulfur) of a sample[3]. This information is important to help determine the structure of an unknown compound, as well as to help ascertain the structure and purity of a synthesized compound.

6.2 Methods

The most common form of elemental analysis, CHN analysis, is accomplished by combustion analysis. In this technique, a sample is burned in an excess of oxygen, and various traps collect the combustion products—carbon dioxide, water, and nitric oxide. The masses of these combustion products can be used to calculate the composition of the unknown sample[4,5].

6.2.1 Combustion analysis

The method was invented by Joseph Louis Gay-Lussac. Justus von Liebig studied the method while working with Gay-Lussac between 1822 and 1824 and improved the method in the following years to a level that it could be used as a standard procedure for organic analysis. Combustion analysis is a method used in both organic chemistry and analytical chemistry to determine the elemental composition (more precisely empirical formula) of a pure organic compound by combusting the sample under conditions where the resulting combustion products can be quantitatively analyzed. Once the number of moles of each combustion product has been determined the empirical formula or a partial empirical formula of the original compound can be calculated. A combustion train is an analytical tool for the determination of elemental composition of a chemical compound. With knowledge of elemental composition a chemical formula can be derived. The combustion train allows the determination of carbon and hydrogen in a succession of steps[6]:

- combustion of the sample at high temperatures with copper oxide as the oxidizing agent,
- collection of the resulting gas in an anhydrous agent (magnesium perchlorate or calcium chloride) to trap generated water,
- collection of the remainder gas in a strong base (for instance potassium hydroxide) to trap generated carbon dioxide.

Analytical determination of the amounts of water and carbon dioxide produced from a known amount of sample gives the empirical formula. For every hydrogen atom in the compound $1/2$ equivalent of water is produced, and for every carbon atom in the compound 1 equivalent of carbon dioxide is produced.

6.2.2 CHNSO Analysis

A CHN Analyzer is a scientific instrument which can determine the elemental composition of a sample. The name derives from the three primary elements measured by the device: carbon (C), hydrogen (H) and nitrogen (N). Sulfur (S) and oxygen (O) can also be measured [7-10]. The analyzer uses a combustion process to break down substances into simple compounds which are then measured.

By separating out inorganic carbon using a solvent, organic carbon in a sample can be measured using this device as well. In this technique the substance under study is combusted under oxygen stream in a furnace at high temperatures. The end products of the combustion would be mostly the oxides of the concerned elements in the form of gases. These are then separated and carried to the detector using inert gases like helium or argon. It is one of the few analytical techniques that give a clear quantitative measurement of the carbon, hydrogen, nitrogen and sulphur. It finds applications in almost every field of chemistry like in the analysis of organics (especially to find out the molecular formula of a newly synthesized compound), polymers, pharmaceuticals, energy (fuels), environmental studies, etc.

As analytical chemistry moves into the 21st century the demands on laboratory staff and equipment to become more productive with less resource and at lower cost are common place. As a consequence of these changes, the time of the analytical specialist is typically becoming increasingly focused on method development and problem solving tasks. This has provided a significant driving force to ensure that routine analyses can be undertaken by any scientist in an open access manner, at any time in a work programme. With a history stretching back to Lavoisier the accurate elemental analysis of organic compounds has long

been a key tool for characterising sample composition and purity in research and quality control environments. The use of CHN microanalysis as a routine technique to provide a precise and accurate elemental analysis of a sample is widely accepted across many industries worldwide. CHN microanalysis is a powerful and relatively straightforward method for determining whether or not a sample is pure by providing a precise and accurate analysis of a sample's percentage carbon, hydrogen and nitrogen content. With the advent of the horizontal furnace design, cross contamination and alteration of the gas flow have effectively been eliminated. This has led to enhanced accuracy and precision and the elimination of memory effects that occur in the vertical furnace configuration. CHN microanalysis has now been developed so that samples can be run unattended in an automatic mode. The demands of accurate weighing of the microsamples has, to date, eluded attempts to replace the scientist with a robotic sample handler. Thus, the main drawbacks of CHN analysis are the requirements for accurate weighing of 1-2 mg of sample (that is consumed in the analysis) and also the fact that samples are usually run as an automated batch rather than on an individual sample basis. Despite these perceived drawbacks, CHN microanalysis remains the most direct purity analysis and so is attractive in the context of increased regulatory pressure and global competition where high quality validatable purity data is essential.

The analysis of results is performed by determining the ratio of elements from within the sample, and working out a chemical formula that fits with those results. This process is useful as it helps determine if a sample sent is a desired compound and confirms the purity of a compound. The accepted deviation of elemental analysis results from

the calculated is 0.4%. The method for working out the ratio of elements from the results is shown below[11]:

1. Take the percentage of each element found and divide by the element's mass. Do this for all the elements for which you have results
2. Find the smallest value from step 1 and divide every value obtained in step 1 by this smallest value
3. Multiply the results in step 2 by a factor to obtain reasonable values for either carbon or nitrogen and then compare to what was expected from a pure sample of the compound that was thought to be submitted

This process is tedious to perform by hand, and automated tools have been released to simplify with this process. Each of the tools is different in its working. `[[CHN+]]` works under Windows and was designed primarily for discovering solvents occluded in in a compound. The Solvent Correction CHN Calculator works in a similar manner, but requires an internet connection. The Chemical Composition Calculator works without an internet connection, calculates elemental analysis on the fly in a user's web browser but predicts molecular ion peaks for use in Mass Spectra. Iteration to discover occluded solvents, is left to the user.

6.3 Experiment



Figure 6.1: C-H-N-S-O Analyzer at SICART, V.V.Nagar, Gujarat

Make Model: Perkin Elmer, U.S.A 2400 Series II

CHNS/O Elemental Analyzer Perkin Elmer PE 2400 Absolute CHN, CHNS, O modes of operations; Accuracy: + 0.3%; 60 samples autosampler, Analysis/estimation of C, H, N, S, O in solid samples.

- Used for determination of Carbon, Hydrogen, Nitrogen, Sulphur and Oxygen contents in organic compounds and their presence if any in inorganic compounds
- Fastest technique to analyze C, H, N, S & O
- The analysis time is around 8 to 10 minutes per sample

- Auto sampler achieves unattended automatic sampling of 60 samples.
- CHNS/O analyzer PE-2400 series II works on frontal chromatography technique for separation and estimation of C, H, N, S and O elements in a sample.
- The analyzer has three mode of operation namely CHN, CHNS and O for respective elemental analysis
- Typical analysis accuracy of 0.3% and analysis time of 8 to 10 minutes per sample

Specifications:

- Analyzed Elements: Carbon, Hydrogen, Nitrogen, Sulfur and Oxygen
- Operating Mode: CHN, CHNS and OXYGEN
- Accuracy: 0.3% abs
- Analysis Time: 6 to 8 minutes per sample
- Automatic Weight Transfer
- Sample Size: 1 to 200 mg
- Multiple Sample Mode if required: 60 samples
- Sample Combustion Temperature: Around 2000 ° c.

Applications:

- Used for knowing the concentration & percentage of C,H,N,S & O in newly synthesized compounds, quality control, metallurgical samples, catalysts, petrochemicals etc depending upon the values of these, products can be identified

- The best solution for the analysis of Organic compounds, Pharmaceuticals, Petrochemicals, Gasoline & Flues, Coal & Coke, Graphite, Carbides & Nitrides, Polymers
- Chemistry and pharmacy Fine chemicals, Medicine, Fertilizers, Catalyst
- Agriculture products Plants and leaves, Wood, Food products, Dairy products, Tobacco

The original analysis method is based on the complete and instantaneous oxidation of the sample by "flash combustion" which converts all organic and inorganic substances into combustion products. The resulting combustion gases pass through a reduction furnace and are swept into the chromatographic column by the carrier gas which is helium. The gases are separated in the column and detected by the thermal conductivity detector which gives an output signal proportional to the concentration of the individual components of the mixture. The results are comparable to those obtained by traditional methods, such as Kjeldahl and Dumas, but it offers faster analysis time with greater reproducibility and accuracy. An extra benefit of the flash combustion method is that no hazardous waste is created other than the spent columns that can be used for hundreds of samples. The sample to be analyzed is weighed into a tin container and loaded into the autosampler. The sample is then deposited into the combustion reactor which is maintained at 1020 degrees C. The sample and container melt and the tin promote a violent reaction in a temporarily enriched atmosphere of oxygen. Under these conditions even thermally resistant substances are completely oxidized. The mixture of combustion products pass first through an oxidation catalyst of chromium trioxide in the reaction/combustion tube. The combustion products, those of interest being CO₂, N₂ and NO_x, along with some water, then pass

through a second tube known as the reduction reactor. It contains metallic copper kept at 650C. The excess of oxygen is removed and at this temperature the nitrogen oxides are reduced to elemental nitrogen, which together with CO₂ and water pass to the magnesium perchlorate tube which removes the water. The helium stream then flows through a chromatographic column separating the nitrogen and carbon. From there the gases flow through the thermal conductivity detector which generates an electrical signal proportional to the concentrations. Analyzing a standard of known composition under the same conditions makes it possible to calibrate the instrument and quantify the content of nitrogen and carbon.

6.4 Results and Discussion

(1) Elemental Analysis Study

The analytical data of the crystals are presented in Table: 6.1 indicates 1:2 stoichiometry. The general equation for the formation of binary crystals is shown as below:



Where M = Cu(II) or Ni(II) , L= Schiff Base

All crystals are colored and possess high decomposition points. All are amorphous and stable in air. The crystals are soluble in acetone and DMF, sparingly soluble in ethanol, insoluble in water and other organic solvents like methanol, benzene, chloroform, carbon tetrachloride.

(2) Molar Conductance Study

The conductivity of crystals was measured in DMF as solvent using conductivity meter model, Systronic 361 μ digital. All the crystals

showed the molar conductance values for 10^{-3}M concentration in range 2 to 78 $\text{ohm}^{-1}\text{cm}^2 \text{mol}^{-1}$. It is suggesting that all crystals are nonelectrolyte in nature[12-14]. The molar conductance values of all the crystals are listed in Table: 6.1

Table: 6.1**Analytical Data and Some Physical Properties of Metal Crystals**

Crystals	Mol. Weight	Analysis of elements (%) found (calculated)			Molar Conductance $\text{Ohm}^{-1} \text{cm}^2 \text{Mol}^{-1}$	D.P. ($^{\circ}\text{C}$)
		C %	H %	N %		
Schiffbase	197	78.93 (79.18)	5.71 (5.58)	7.13 (7.10)	-	>250
Cu(II) Crystal	312.5	49.92 (50.90)	3.60 (4.80)	4.50 (4.48)	8.58	>300
Ni(II) Crystal	451.7	78.93 (79.18)	5.71 (5.58)	7.13 (7.10)	12.40	>300

D.P. =Decomposition point

References

1. Bhim B. Prasad, Anil Kumar, Meenakshi Singh, Sandhya Singh(1995)
Electrolytic Conductivity of Crystal Violet-Based Quaternary Ammonium Polyelectrolytes in Propylene Carbonate and Sulfolane
J. Chem. Eng. Data, 40 (1), pp 79–82
2. Xiangming He, WeihuaPu, Jingli Han, Jian Chen, Jiufang Lu, Changyin Jiang, and Chunrong Wan(2005)
Ionic Limiting Molar Conductivity Calculation of Li-Ion Battery Electrolyte Based on Mode Coupling Theory
J. Phys. Chem. B, 109 (49), pp 23141–23144
3. Robinson R. A. and Stokes R. H. (1965)
"Electrolyte Solutions," 2d ed. (revised), Academic Press, Inc., New York.
4. Shedlovsky T. (1960)
Conductometry, in A. Weissberger (ed.), "Technique of Organic Chemistry," vol. 1, "Physical Methods of Organic Chemistry," 3d ed., pt. 4, chap. 45, Interscience Publishers, Inc., New York.
5. Spiro M. (1960)
Determination of Transference Numbers, in A. Weissberger (ed.), "Technique of Organic Chemistry," vol. 1, "Physical Methods of Organic Chemistry," 3d ed., pt. 4, chap 46, Interscience Publishers, Inc., New York.
6. MacInnes D. A. (1939)
"The Principles of Electrochemistry," Reinhold Publishing Corporation New York.

7. Kenneth R. H.(2009)
The fractional Stokes–Einstein equation: Application to Lennard-Jones, molecular, and ionic liquids
J. Chem. Phys. 131, 054503 .
8. González-Pérez A., Del Castillo J. L., Czapkiewicz J. and Rodríguez J. R.(2001)
Temperature Dependence of Equilibrium and Transport Properties of Decyldimethylbenzylammonium Chloride in Aqueous Solutions
J. Chem. Eng. Data, 46 (3), pp 709–711
9. Adams S. and Swenson J.
Predictability of ion transport properties from the structure of solid electrolytes
Chemistry And Materials Sciencelonics Volume 10, Numbers 5-6, 317-326
10. Aleshin A. N., Mironkov N. B. and Suvorov A. V. (1996)
Transport properties of ion-implanted and chemically doped polyaniline films
Phys. Rev. B 54, 11638–11643.
11. Oak Ridge(1981)
A generalization of the nernst-Einstein equation for self-diffusion in high defect concentration solids
Solid State Ionics Volume 5, October , Pages 133-136
12. Geary W.J.(1971)
“The use of conductivity measurements in organic solvents for the characterization of coordination compounds”
Coord.Chem. Rev., 7, 81.
13. Bauer A.W., Kirby W.M.M., Sherris J.C. and Turck M.(1966)
“Antibiotic susceptibility testing by a standardized single disk method”
Amer. J. Clin. Pathol., 45(6), 493.

14. Jian G.(2007)

“A weak hydroticalCu(II) complex derived from condensation of N,N-dimethylbiguanide with 2-pyridine carbaldehyde synthesis, crystal structure and biological activity”

Synth. And React. In Inorg.Metal-Org. and Nano-Met.Chem., 37, 621.