09 ANALYTICAL INSTRUMENTATION – WORKING PRINCIPLES AND APPLICATIONS IN MARINE ENVIRONMENT

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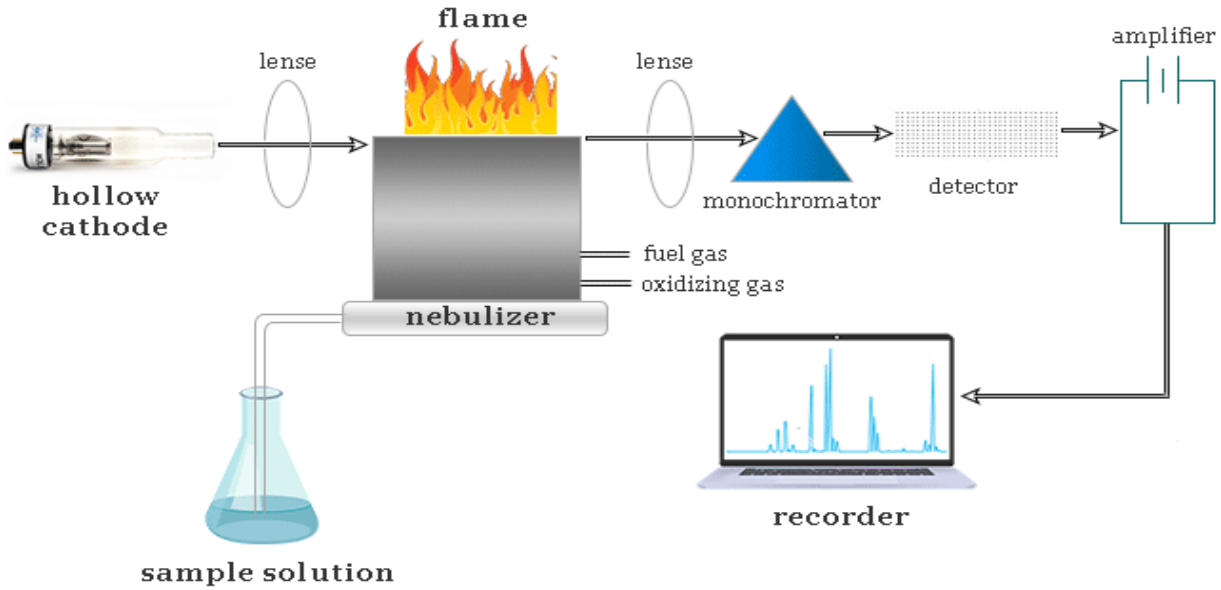
I. UV VISIBLE SPECTROPHOTOMETER

UV Visible spectrophotometer measures the response of a sample to ultraviolet and visible range of electromagnetic radiations. It operates by passing a beam of light through a sample and measuring the intensity of light reaching the detector. A spectrophotometer consists of two main components, namely a spectrometer for producing light of any selected wavelength, and a photometer for measuring the intensity of light. The instruments are designed in such a way that that liquid in a cuvette can be placed between the spectrometer beam and the photometer. The amount of light passing through the cuvette is measured by the photometer. The signal changes as the amount of light absorbed by the liquid changes. According to Beer's law, when light of a specific wavelength passes through a solution there is usually a quantitative relationship between the solute concentration and the intensity of the transmitted light. The O.D. value recorded is directly proportional to the concentration of the coloured compound. Most spectrophotometers have a scale that reads both in O.D. (absorbance) units, which is a logarithmic scale, and in % transmittance, which is an arithmetic scale. As suggested by the above relationships, the absorbance scale is the most useful for colorimetric assays. The applications of spectrophotometer include water quality assessment and other quantitative and qualitative analysis, enzyme assays, turbidimetry, nephelometry etc

II. ATOMIC ABSORPTION SPECTROPHOTOMETER

Atomic absorption spectroscopy (AAS) is a precise analytical technique used for quantification of metals present in the analyte. This technique involves measuring the absorption of radiant energy (usually ultraviolet and visible) by the free atoms of the metal of interest.



When an atom of the metal of interest absorbs light of a specific wavelength, it results in the excitation of electrons from the ground state to the excited state. The energy absorbed per mole is fixed and can be used for qualitative and quantitative analysis. AAS uses the absorption of light to measure the concentration of gas-phase atoms in the analyte. Since samples are usually liquids or solids, the analyte atoms or ions must be vaporized in a flame or graphite furnace. The analyte concentration is determined from the amount of absorption. The relationship between absorbance and concentration of an absorbing species is explained by Beer-Lambert law (or Beer's law). Every AAS instrument must have 3 basic components; (1) a light source; (2) a sample compartment; and (3) a detector.

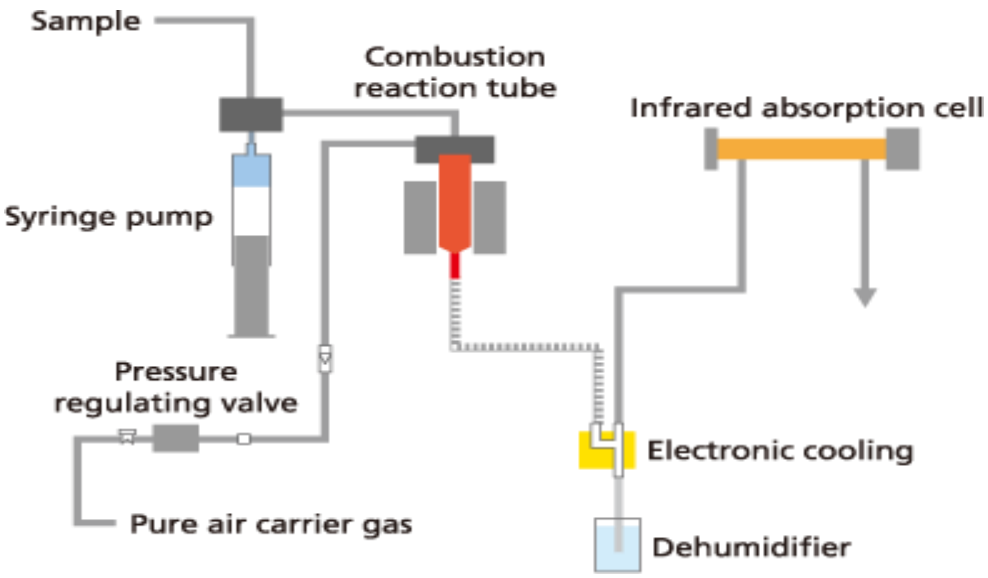
III. CHNS ANALYSER

CHNS elemental analysers provide a means for the rapid determination of carbon, hydrogen, nitrogen and sulphur in water or sediment samples. The applications of CHNS includes protein analysis in fish and other marine products. Measuring the total content of N, C and S in water and sediment samples also plays an important role in understanding the level of pollution and nutrient dynamics in marine ecosystems. The basic principle of operation is high temperature digestion of the sample at 800°C to 1200°C in a pure oxygen environment, with subsequent scrubbing of non-analyte from the combustion gases. In the combustion process, carbon is converted to carbon dioxide; hydrogen to water; nitrogen to nitrogen gas/ oxides of nitrogen and sulphur to sulphur dioxide. If other elements such as chlorine are present, they will also be converted to combustion products, such as hydrogen chloride. A variety of absorbents are used to remove these additional combustion products. The combustion products are swept out of the combustion chamber by inert carrier gas such as helium and passed over heated high purity copper. The function of this copper is to remove any oxygen not consumed in the initial combustion and to convert oxides of nitrogen to nitrogen gas. The gases are then passed through the absorbent traps in order to leave only carbon dioxide, water, nitrogen and sulphur dioxide. Detection of the gases are carried out by GC separation followed by quantification using thermal conductivity detection. Quantification of the elements requires calibration for each element by using high purity 'micro-analytical standard' compounds such as acetanilide and benzoic acid.

IV. TOC ANALYSER

The carbon present in the sample is digested first by the Total Organic Carbon Analyser (TOC) into carbon dioxide and the formed CO_2 is brought to detectors that can detect carbon dioxide at very low levels. The analyser can be used to determine carbon levels from different sorts of water and sediment samples. The steps involved in TOC measurement includes the measurement of total carbon (TC) using one of the methods of destruction of carbon, measurement of total inorganic carbon (TIC) by acidification of the sample and subtraction of TIC from TC results to get the total organic carbon (TOC). It is also possible to remove the inorganic carbon from the sample first and the left-over TOC can be measured directly. This method comprises purging an acidified sample prior to measurement and so is more accurately called Non- purgeable organic carbon (NPOC). Total organic carbon analysis by TOC analysers is divided into acidification, oxidation, detection and quantification stages.

In general, the TOC is determined by oxidizing the sample. The produced CO_2 is detected and defined quantitatively. However, not all methods succeed in the complete oxidation of a sample. The combustion catalytic oxidation method is commonly used worldwide. One of the most important features of this method is the capacity to efficiently oxidize hard-to-decompose organic compounds, including insoluble and macromolecular organic compounds. The carbon dioxide generated by oxidation is detected using an infrared gas analyzer (NDIR).



Schematic Diagram of the TOC Analyzer

Autoanalyzer

Water analysis using conventional methods such as the Strickland and Parsons method often require significant time and resources, and their accuracy can be influenced by the practitioner's skill and experience. Automating the analysis can increase the efficiency of laboratory operations, improve sample throughput, enhance accuracy, eliminate human errors, and reduce costs in general, such as operator time, analysis speed, reagent consumption, and waste. Automatic analyzers based on segmented flow technology are generally considered cost-effective and productive. Segmented flow analysis is a technique used in analytical chemistry for automating chemical analyses. It involves dividing a continuous flow of liquid (Sample+reagent) into discrete segments using air bubbles. This segmentation allows for precise control of the reaction time and conditions, leading to improved accuracy and precision. Thus automatic segmented flow analysis is a continuous flow method of wet chemistry analysis in which a stream of reagents and samples, segmented with air bubbles, is pumped through a manifold to undergo treatment such as mixing, heating, dialysis, incubation etc before entering a flow cell to be detected. After color development at the chemistry module the liquid flows through a flow cell and the absorbance are measured with aid of an interference filter. The auto analyser currently available in our lab is used for the analysis of nutrients in water, including Nitrite, Nitrate, Ammonia, Silicate, and Phosphate. The latest technologies are integrated into the most versatile range of auto sampling, innovative reagent switching and accurate and reliable pumping, for more than a thousand proven chemistry applications.

The basic steps in autoanalyzer includes

a) **Sample Introduction:** The sample is introduced into a flowing stream of carrier liquid.

b) **Segmentation:** Air bubbles are injected into the stream, dividing it into segments.

c) **Reagent Addition:** Reagents necessary for the analysis are added to each segment.

d) **Incubation:** The segments are allowed to react for a specified time.

e) **Detection:** The reaction products are measured using a suitable detector (e.g., spectrophotometer, fluorometer).

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