COURSE CODE:	ANN 509
COURSE TITLE:	Instrumentation and Feed Analysis
NUMBER OF UNITS:	2 Units
COURSE DURATION:	4 Hours (2 hours lecture and 2 hours practical)

COURSE DETAILS:

Course Coordinator:	**Dr. Olubukola Ajike Isah**
Email:	**bukkyisah@yahoo.com**
Office Location:	Department of Animal Nutrition
Other Lecturers:	**Prof. Daisy Eruvbetine, Dr. Adebayo Vincent Jegede, Dr. Adebayo Olusoji Oni**

COURSE CONTENT:

Basic principles governing common laboratory equipment, principles governing basic analytical procedures.

COURSE REQUIREMENTS:

This course is expected to be taken by all final year students of College of Animal Science and Livestock Production. This course is being offered in lecture format on-campus for 14 weeks, students are expected to attend lectures and practical classes, 2 hours of lecture per week. Students are expected to have a minimum of 75% attendance to be able to write the final examination.

The course grade will be based on 1 exams, CAT and practical work.

READING LIST:

Basic Animal Nutrition and Feeding, W.G. Pond, D.C. Church and K.R. Pond, Wiley, Fourth Edition, 1995

Analytical Agricultural Chemistry, S.L. Chopa and J.S. Kanwar,1st Edition Kayani Publisher , New Delhi - Ludhiana

LECTURE NOTES

PROXIMATE ANALYSIS OF FEEDSTUFF

Sampling and Preparation for Analysis

Before undertaking an analysis the results of which are to be used to represent the composition of a consignment of a feedstuff, it is important that the sample is sufficient in amount and that it is selected properly from the bulk so as to be fairly representative of it. Sampling is however not a laboratory operation. The sample to be used, if necessary, is expected to be sufficiently dried to enable it to be finely ground.

PROXIMATE ANALYSIS

This refers to the determination of the major constituents of feed and it is used to assess if a feed is within its normal compositional parameters or somehow being adulterated. This method partitions nutrients in feed into 6 components: water, ash, crude protein, ether extract, crude fibre and NFE.

Moisture Determination

Moisture is determined by the loss in weight that occurs when a sample is dried to a constant weight in an oven. About 2g of a feed sample is weighed into a silica dish previously dried and weighed. The sample is then dried in an oven for 65^0C for 36 hours, cool in a desiccator and weighed. The drying and weighing continues until a constant weight is achieved.

$$\%\text{Moisture} = \frac{\text{wt of sample + dish before drying - wt of sample+ dish after drying}}{\text{Wt of sample taken}} \times 100$$

Since the water content of feed varied widely, ingredients and feed are usually compared for their nutrient content on moisture free or dry matter (DM) basis.

%DM = 100 - %Moisture.

Ether Extract

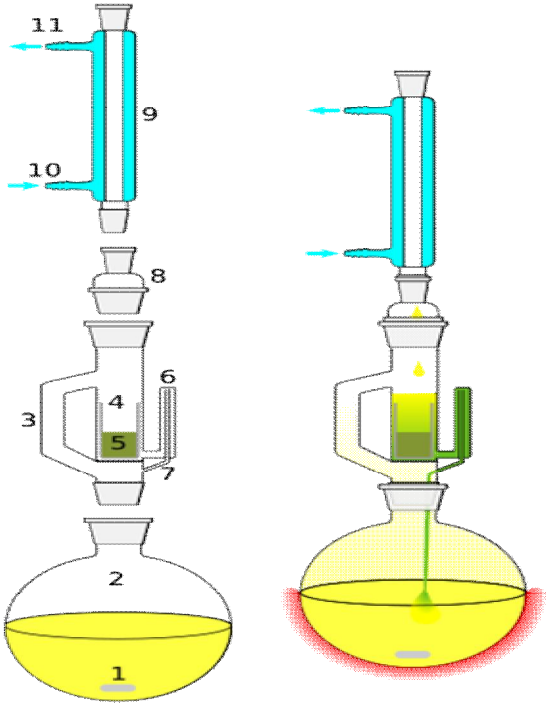
The ether extract of a feed represents the fat and oil in the feed. **Soxhlet apparatus** is the equipment used for the determination of ether extract. It consist of 3 major components

1. An extractor: comprising the thimble which holds the sample
2. Condenser: for cooling and condensing the ether vapour
3. 250ml flask

Procedure: about 150ml of an anhydrous diethyl ether (petroleum ether) of boiling point of 40-60^{0}C is placed in the flask. 2-5g of the sample is weighed into a thimble and the thimble is plugged with cotton wool. The thimble with content is placed into the extractor; the ether in the flask is then heated. As the ether vapour reaches the condenser through the side arm (distillation path) of the extractor, it condenses to liquid form and drop back into the sample in the thimble, the ether soluble substances are dissolved and are carried into solution through the syphon tube back into the flask. The extraction continues for at least 4 hrs. The thimble is removed and most of the solvent is distilled from the flask into the extractor. The flask is then disconnected and placed in an oven at 65^{0}C for 4 hrs, cool in desiccator and weighed.

$$\%\text{Ether extract} = \frac{\text{wt of flask + extract – tare wt of flask}}{\text{wt of sample}} \times 100$$

Soxhlet Apparatus



1: *Stirrer bar/anti-bumping granules* ***2:*** *Still pot (extraction pot) - still pot should not be overfilled and the volume of solvent in the still pot should be 3 to 4 times the volume of the soxhlet chamber.* ***3:*** *Distillation path* ***4:*** *Soxhlet Thimble* ***5:*** *Extraction solid (residue solid)* ***6:*** *Syphon arm inlet* ***7:*** *Syphon arm outlet* ***8:*** *Expansion adapter* ***9:*** *Condenser* ***10:*** *Cooling water in* ***11:*** *Cooling water out*

Crude Fibre

The organic residue left after sequential extraction of feed with ether can be used to determine the crude fibre, however if a fresh sample is used, the fat in it could be extracted by adding petroleum ether, stir, allow it to settle and decant. Do this three times. The fat-free material is then transferred into a flask/beaker and 200mls of pre-heated 1.25% H_2SO_4 is added and the solution is gently boiled for about 30mins, maintaining constant volume of acid by the addition of hot water. The buckner flask funnel fitted with whatman filter is pre-heated by pouring hot water into the funnel. The boiled acid sample mixture is then filtered hot through the funnel under sufficient suction. The residue is then washed several times with boiling water (until the residue is neutral to litmus paper) and transferred back into the beaker. Then 200mls of pre-heated 1.25% Na_2SO_4 is added and boiled for another 30mins. Filter under suction and wash thoroughly with hot water and twice with ethanol. The residue is dried at 65^0C for about 24hrs and weighed. The residue is transferred into a crucible and placed in muffle furnace (400-600^0C) and ash for 4hrs, then cool in desiccator and weigh.

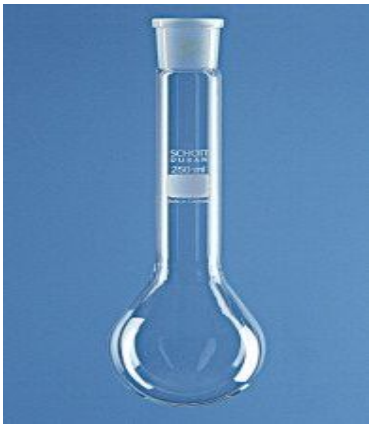
$$\%\text{Crude fibre} = \frac{\text{Dry wt of residue before ashing - wt of residue after ashing}}{\text{wt of sample}} \times 100$$

Crude Protein

Crude protein is determined by measuring the nitrogen content of the feed and multiplying it by a factor of 6.25. This factor is based on the fact that most protein contains 16% nitrogen. Crude protein is determined by **kjeldahl method.** The method involves: Digestion, Distillation and Titration.

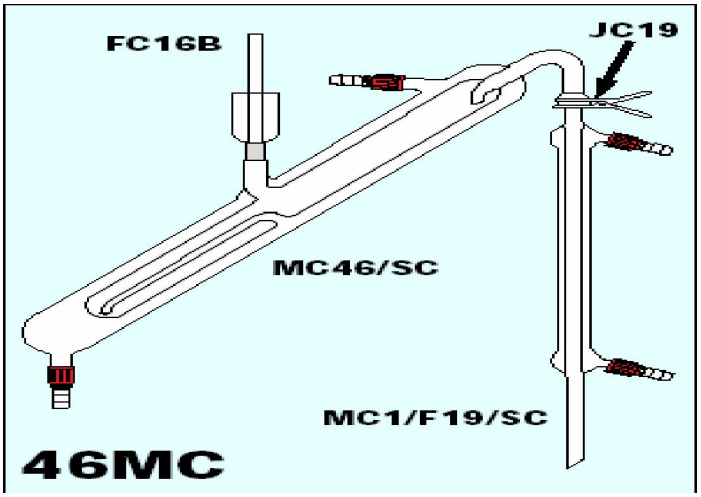
Digestion: weigh about 2g of the sample into kjeldahl flask and add 25mls of concentrated sulphuric acid, 0.5g of copper sulphate, 5g of sodium sulphate and a speck of selenium tablet. Apply heat in a fume cupboard slowly at first to prevent undue frothing, continue to digest for 45mins until the digesta become clear pale green. Leave until completely cool and rapidly add 100mls of distilled water. Rinse the digestion flask 2-3 times and add the rinsing to the bulk.

Kjeldahl Flask



Distillation: Markham distillation apparatus is used for distillation. Steam up the distillation apparatus and add about 10mls of the digest into the apparatus via a funnel and allow it to boil. Add 10mls of sodium hydroxide from the measuring cylinder so that ammonia is not lost. Distil into 50mls of 2% boric acid containing screened methyl red indicator.

Markham Distillation Apparatus



Titration: the alkaline ammonium borate formed is titrated directly with 0.1*N* HCl. The titre value which is the volume of acid used is recorded. The volume of acid used is fitted into the formula which becomes

$$\%N = \frac{14 \times VA \times 0.1 \times w \quad x100}{1000 \times 100}$$

VA = volume of acid used w= weight of sample

%crude protein = %N x 6.25

Ash

Ash is the inorganic residue obtained by burning off the organic matter of feedstuff at 400-600^{0}C in muffle furnace for 4hrs. 2g of the sample is weighed into a pre-heated crucible. The crucible is placed into muffle furnace at 400-600^{0}C for 4hrs or until whitish-grey ash is obtained. The crucible is then placed in the desiccator and weighed

$$\%Ash = \frac{\text{wt of crucible+ash} - \text{wt of crucible}}{\text{wt of sample}}$$

Nitrogen Free Extract (NFE)

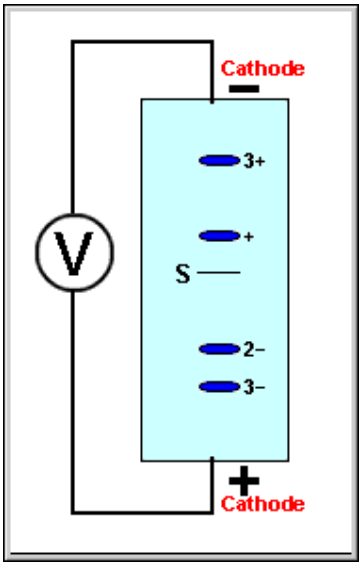
NFE is determined by mathematical calculation. It is obtained by subtracting the sum of percentages of all the nutrients already determined from 100.

%NFE = 100-(%moisture + %CF + %CP + %EE + %Ash)

NFE represents soluble carbohydrates and other digestible and easily utilizable non-nitrogenous substances in feed.

ELECTROPHORESIS

Electrophoresis is a separation technique that is based on the mobility of the ions in an electric field.. it is a Greek word Electros meaning “energy from an electric field and Phoros meaning to “carry across”. Thus electrophoresis means the techniques in which molecules are forced across a span of gel motivated by an electric field



Electrophoresis is a technique whereby charged molecules are separated on the basis of their speed of migration in an applied electric field. Molecules that are cationic (that is, are positively charged) will migrate towards the negatively charged electrode (the cathode), while those molecules that are anionic (that is, are negatively charged) will migrate towards the positive electrode (the anode). The higher the charge, the faster will a molecule move towards the oppositely charged electrode.

If a mixture of charged molecules are placed in the center of a supporting medium (at the point S in the diagram on the right), and the electric field set up by means of an applied potential, **V**, then, after a while, separation of the molecules will take place. It similar to chromatography in many ways except that separation is due to movement of charged particles through an electrolyte (buffer when subjected to electric current.

Types of Electrophoresis

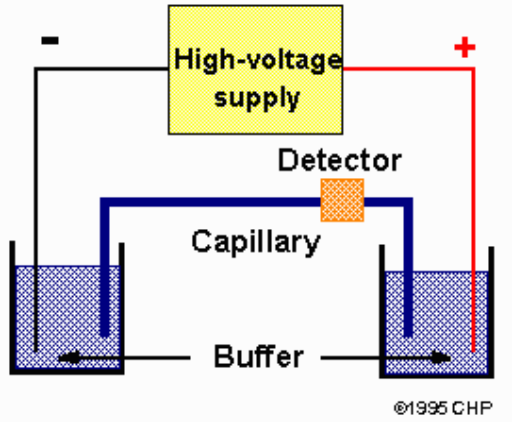
Capillary Electrophoresis

Capillary electrophoresis (CE) encompasses a family of related separation techniques that use narrow-bore fused-silica capillaries to separate a complex array of large and small molecules. High electric field strengths are used to separate molecules based on differences in charge, size and hydrophobicity.

Capillaries are typically of 50 µm inner diameter and 0.5 to 1 m in length. The applied potential is 20 to 30 kV. Due to electroosmotic flow, all sample components migrate towards the negative electrode. A small volume of sample (10 nL) is injected at the positive end of the capillary and the separated components are detected near the negative end of the capillary. CE detection is similar to detectors in HPLC, and includes absorbance, fluorescence, electrochemical, and mass spectrometry.

The capillary can also be filled with a gel, which eliminates the electroosmotic flow. Separation is accomplished as in conventional gel electrophoresis but the capillary allows higher resolution, greater sensitivity, and on-line detection.

Schematic of capillary electrophoresis



Gel Electrophoresis

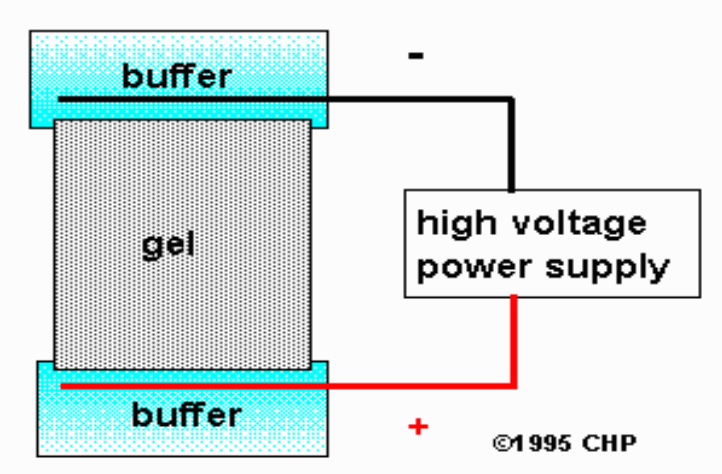
Gel electrophoresis is a technique used for the separation of deoxyribonucleic acid, ribonucleic acid, or protein molecules using an electric current applied to a gel matrix. It is usually performed for analytical purposes, but may be used as a preparative technique prior to use of other methods such as mass spectrometry. In most cases the gel is a crosslinked polymer whose composition and porosity is chosen based on the specific weight and composition of the target to be analyzed. When separating proteins or small nucleic acids (DNA, RNA, or oligonucleotides) the gel is usually composed of different concentrations of acrylamide and a cross-linker, producing different sized mesh networks of polyacrylamide. When separating larger nucleic acids (greater than a few hundred bases), the preferred matrix is purified agarose. In both cases, the gel forms a solid, yet porous matrix. Acrylamide, in contrast to polyacrylamide, is a neurotoxin and must be handled using Good Laboratory Practices to avoid poisoning. By placing the molecules in wells in the gel and applyng an electric current, the molecule will move through the matrix at different at different rates usally determined by mass toward the positive (Anode) if negatively charged or toward the negative (Cathode) if positively charged.

After the electrophoresis is complete, the molecules in the gel can be stained to make them visible. Ethidium bromide, silver, or coomassie blue dye may be used for this process. Other methods may also be used to visualize the separation of the mixture's components on the gel. If the analyte molecules fluoresce under ultraviolet light, a photograph can be taken of the gel under ultraviolet lighting conditions. If the molecules to be separated contain radioactivity added for visibility, an autoradiogram can be recorded of the gel.

If several mixtures have initially been injected next to each other, they will run parallel in individual lanes. Depending on the number of different molecules, each lane shows separation of the components from the original mixture as one or more distinct bands, one band per component. Incomplete separation of the components can lead to overlapping bands, or to indistinguishable smears representing multiple unresolved components.

Bands in different lanes that end up at the same distance from the top contain molecules that passed through the gel with the same speed, which usually means they are approximately the same size. There are molecular weight size markers available that contain a mixture of molecules of known sizes. If such a marker was run on one lane in the gel parallel to the unknown samples, the bands observed can be compared to those of the unknown in order to determine their size. The distance a band travels is approximately inversely proportional to the logarithm of the size of the molecule.

Schematics of Gel Electrophoresis



Application

Gel electrophoresis is used in forensics, molecular biology, genetic and biochemical analysis.

GEL

Gel is a solid, jelly-like material that can have properties ranging from soft and weak to hard and tough. Gels are defined as a substantially dilute crosslinked system, which exhibits no flow when in the steady-state.[1] By weight, gels are mostly liquid, yet they behave like solids due to a three-dimensional crosslinked network within the liquid. It is the crosslinks within the fluid that give a gel its structure (hardness) and contribute to stickiness (tack).

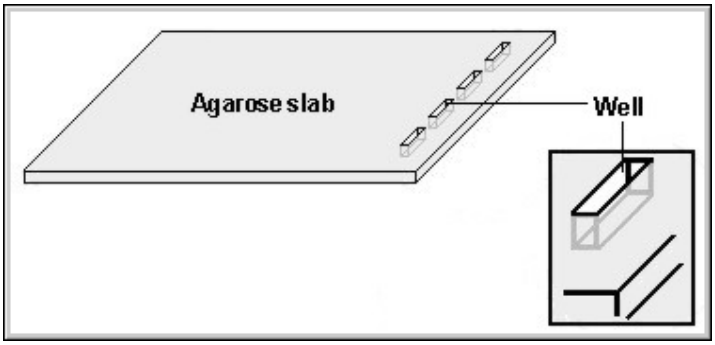
Gel electrophoresis is commonly carried out on a slab of agarose. The dry substance is allowed to swell in hot buffer solution, and cast into a mould, which leaves small "wells" in the gel, into which the samples are applied. Other media that are used as support are: paper, cellulose acetate, starch, or polyacrylamide. For the latter, small slabs are prepared and run vertically in specially designed apparatus, to be described later.

The velocity, **v**, will also depend on the resistance to the movement of the molecules provided by the matrix through which the molecules are moving. Thus, the type of support that is used is very important. Agarose gels of various concentrations may be prepared by altering the ratio of dry agarose to the buffer. Typically, agarose gels are used in a concentration varying between 0.5% and 2%. Since molecular sieving takes place to varying extents, the more concentrated the gel, the slower the mobility of the molecules in the same buffer and applied potential difference.

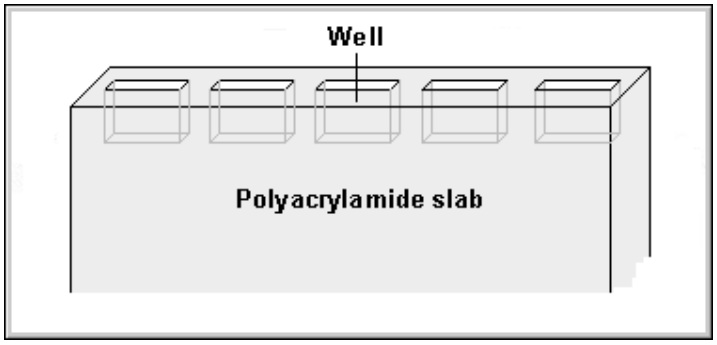
Agarose gels are normally used to separate native proteins, that is, proteins that have retained higher orders of structure. One frequently refers to such gels as "native gels". Separation on native gels takes place by both charge AND size. Polyacrylamide gels are normally used in conjunction with sodium docecyl sulphate.

Two major materials used in making gels are-:

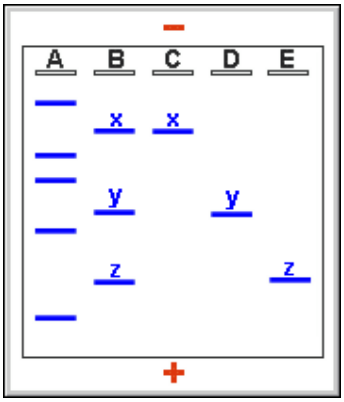
1. **Agarose –** One of the materials used in electrophoresis. It is extracted in the form of agar from several species of red marine algae, or seaweed. It is highly fragile and can easily destroyed by handling. Agarose gels have a very large pore size and are used primarily to separate molecules (large molecular mass). It can be processed faster than polyacrylamides gels but their resolution is inferior. The band formed are usually far apart. Agarose is a linear polysaccharide made up of basic agarobiose unit which comprise of alternate unit of galactose and anhydrogalactose. It is usually used between 1% and 3%.



2. **Polyacrylamide –** Polyacrylamide gels can be used to provide varieties of electrophoretic condition. It is currently most often used in the field of immunology and protein analysis, often used to separate different proteins or isoforms of the same protein into separate bands. The pore size can be varied so as to produce different molecular sieving for protein of different sizes. Polyacrylamide gels offer greater flexibility and more sharply defined banding than agarose gel.



Polyacrylamide gels are made by polymerising acrylamide monomer ( ) with ammonium persulphate ( ) in the presence of N,N'-methylene-bisacrylamide ( ) ("bis crosslinker").



The resulting gel consists of minute "tunnels" of various diameters, which can selectively accomodate the passage of molecules, based on their sizes. There exists a linear relationship between the distance

travelled in a given time under defined conditions and the logarithm of the molar mass of the proteins (diagram above, left). This is exploited in determining the molar mass of samples. The migration distance of unknown proteins is simply related to that of "markers" of known molecular masses. A schematic representation of this is shown in the diagram above on the right, where protein samples are electrophoresed in five "lanes" **A-E**, all migrating towards the positive pole of the electrophoretic cell:

Lane **A** consists of protein markers. Lane **B** consists of a mixture three proteins: **x** being the largest, and **z** the smallest, with **y** having a size intermediate between the two. Lane **C**, has pure protein **x**, lane **D** has pure **y** and lane **E** pure **z**.

Electrophoresis Using Acetic Strips

Cellulose acetate electrophoresis nowadays plays an important role in clinical diagnostics routine procedures but has also helped in investigating a broad range of subjects in life science research.

Cellulose acetate electrophoresis separates proteins primarily by charge. Protein migration takes place on buffer film on the surface of the cellulose acetate paper.

Application

Analysis of haemoglobin is a typical example. Also in separation of enzyme (creatinine, phospokinase, GOT, acidic erythrocyte, phosphatase, phosphoglucomutase etc), mucopolysaccharide, plasma serum, cerebro-spinal fluid, urine and other body fluids. Another field of application is in quality control of biological compounds. It is an accurate simple method of protein quantification.

Radioactivity

Radioactivity is the spontaneous disintegration of atomic nuclei. The nucleus emits **α** particles, **β** particles, or electromagnetic rays during this process. Radioactivity is the process whereby unstable atomic nuclei release energetic subatomic particles. The word *radioactivity* is also used to refer to the subatomic particles themselves. This phenomenon is observed in the heavy elements, like uranium, and unstable isotopes, like carbon-14.

For a better understanding of radioactivity, a review of atomic structure is necessary.

Atoms are made up of a dense positive core called a nucleus surrounded by orbiting electrons. Nucleus contains protons (carry + charge) and neutron which is as heavy as proton but zero charged. Electrons are light and have negative.

Isotopes of elements contain nuclei with same number of protons but different number of neutrons. Atom where the number of protons does not equal to the number of neutrons is unstable.

The chemical properties of an atom are determined by the number of protons in the nucleus e.g every atom which has 6 protons in its nucleus is a carbon atom. Different numbers of neutrons may exist in a carbon nucleus; there can be 5, 6, 7 or 8. Each of these atoms is a different isotope of carbon.

All elements have isotope(s). Some isotopes are stable and some are unstable. An unstable atom has too many neutrons in its nucleus. To get rid of the excess, the nucleus decays into different nucleus by throwing out (emitting) particles and energy.

Whenever there is a disintegration of atomic nuclei, the nucleus emits **α** particles, **β** particles, or electromagnetic rays during this process

Alpha (α) Decay

Alpha particles (named after and denoted by the first letter in the Greek alphabet, α) consist of two protons and two neutrons bound together into a particle identical to a helium nucleus, which is produced in the process of alpha decay. The alpha particle can be written as He^{2+}, $_{42}He^{2+}$ or $_{42}He$

.So when an atom undergoes **α** decay, its atomic number decreases by 2 and its atomic mass decreases by 4. An example of **α** decay is the following

Pu239 ⟶ U235 + **α** particle (He-4 nucleus)

They are a highly ionizing form of particle radiation, and (when resulting from radioactive alpha decay) have low penetration depth. They are able to be stopped by a few centimeters of air, or by the skin.

$^{238}_{92}U \rightarrow {}^{234}_{90}Th + {}^{4}_{2}He^{2+}$ [1]

Or:

$^{238}_{92}U \rightarrow {}^{234}_{90}Th + {}^{4}_{2}He$

$^{238}U \rightarrow {}^{234}Th + \alpha$

Beta (β) Decay

There 2 types of **β** decay; β^+ and β^- decay.

β^- Decay : An excess of neutrons in an atom's nucleus will make it unstable, an a neutron is converted into a proton to change this ratio. During this process, a **β** particle is released, and it has the same mass and charge as an electron. The resulting atom and the **β** particle have a total mass which is less than the mass of the original atom. The atomic number of the atom increases by 1

$^{137}_{55}Cs \longrightarrow {}^{137}_{56}Ba + e^-$

β^+ Decay: When there is an excess of protons in the nucleus, and it is not energetically possible to emit a particle, β^+ Decay occurs. This is where the nucleus becomes stable by converting a proton into a neutron. During β^+ decay, a positron (a particle with the same mass as an electron but with positive charge) and a neutrino are released. Positrons interact with electrons, causing both to be completely destroyed. 2 gamma ray photons with same energy as the mass of the positron and electron are released.

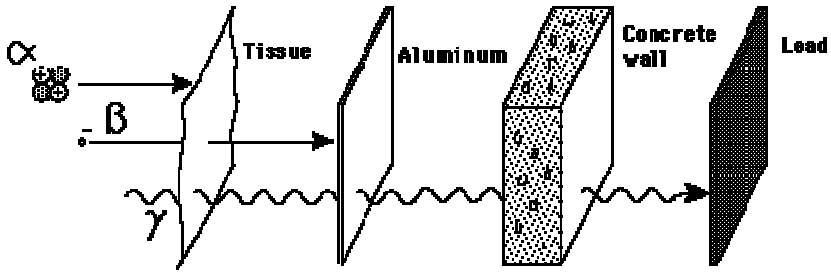
$^{22}_{11}Na \longrightarrow {}^{22}_{10}Ne + e^+$

Gamma Radiation (γ)

Gamma ray emission usually occurs with α and β emission. γ rays have no charge or mass, so their emission doesn't change the chemical composition of the atom. Instead, it results in a loss of radiant energy. Gamma ray emission occurs because the nucleus is often unstable after α and β decay. The emission of α and β particles frequently leaves the product nucleus in an excited state. The excited nucleus can relax back to its ground state by emitting a photon.

Penetration of Matter

Though the most massive and most energetic of radioactive emissions, the alpha particle is the shortest in range because of its strong interaction with matter. The electromagnetic gamma ray is extremely penetrating, even penetrating considerable thicknesses of concrete. The electron of beta radioactivity strongly interacts with matter and has a short range.



Half Life (T½)

A sample of radioactive substance will decay into various particles. The rate of decay is measured by how long it takes for half the sample to decay. The decay of an individual atom is totally random, but for a large sample size, we can get a good prediction of the half life.

Application of Radioisotope to Animal Nutrition Research

An isotopic racer technique is a useful tool in biological study to monitor the movement of nutrients through digestion, absorption and excretion.

- Radio isotopes are useful in studying volume in animal system e.g estimation of plasma volume and rumen fluid in cattle. This is known as isotopic dilution techniques.
- It can be used to test the efficiency of a particular nutrient in diet.
- It can be used to monitor the distribution of elements and compounds to various organs and tissues of the body.
- The measurement of radioactive isotopic potassium makes it possible to estimate the amount of lean meat in animal body.
- It is used to determine substrate and product relationship i.e to determine if substrate A is converted to product B within the animal.
- It is also used in the analysis of rate of processes e.g a body store of particular substances can be labeled through administration of an isotopic tracers and the disappearance of the tracers subsequently monitored.

COLORIMETRY

Colorimetry is the determination of the concentration of a substance by measurement of relative assumption of light or transmitters with respect to a known concentration of the substance. When a beam of radiant energy falls upon a substance, the energy of the beam is partially altered by reflection, refraction, diffraction or absorption and the remaining every may be transmitted through the substance.

FUNDAMENTAL LAWS OF COLORIMETRY

Spectro-colorimetric and photometric principles are based upon Lambert law and Beers law.

a). **LAMBERT LAW -** It states that when monochromatic light passes through a transparent medium; the rate of decrease of monochromatic light (radiation of a single frequency) intensity with the thickness of the medium is proportional to the intensity of the light. This is equivalent to stating

that the intensity of emitted light decreases exponentially as the thickness of absorbing medium increases automatically; or that any layer of given thickness of medium absorbs the same fraction of the light incident upon it.

b). **BEER'S LAW –**Thus, according to Beer's law, the intensity of a beam of monochromatic light decreases exponentially as the concentration of the absorbing substance increases arithmetically.

METHODS OF DIRECT MEASUREMENT OF LIGHT INTENSITY

(I) Klett Summerson Colorimeter

(II) Bausch and Lomb Spectronic Colorimeter

(III) Beckman's DU-Ultra Violet Visible Spectrophotometer

FLAME PHOTOMETER

The instrument is used for detection of metal salts such as Na, K, Li, Ca and Ba level in the sample. It is a simple, relatively inexpensive, high sample throughput method used for clinical, biological and environmental analysis. The source of light is from gas flame. The flame vaporizes the liquid sample into a gaseous state. The compound decomposes into simple molecules or atoms which then get excited to emit light at the temperature of the flame. The flame photometer has the following parts:

1. Pressure regulator
2. Atomizer
3. Optical arrangement
4. Burner
5. Photo-sensitive detector
6. Recording and output device

The set-up is provided with a pressure guage so that the rate of (oxygen) can be regulated.

1. **Pressure regulator:** this indicates the pressure and flow rate that prevails when the instrument is in operation.
2. **Atomizer:** Introduces the liquid sample into the flame at a stable and reproducible rate i.e. every drop that falls must be uniform.
3. **The burner:** the fuel gas should produce a steady flame in the presence of oxygen or air at a constant pressure.
4. **The optical arrangement:** this consists of light which photosensitive device concave mirror is fixed so that the flame is focused properly.

5. **Photosensitive Detector:** It is composed of a metal plate of iron on which it is deposited on thin layer of selenium which acts as semi-conductor. Radiation falls on the semi-conductor and measured on a meter after amplification.

CHROMATOGRAPHY

This is a separation process used for the separation of molecular mixture. It is carried out by mechanical manipulation depending on the physical properties;

1. Solubility – ability to dissolve in liquid
2. Adsorption – ability to attach itself to finely divided solid.
3. Volatility – ability to pass into vapour

Every chromatography separation has a "stationary phase" which has a packing within a column and a "mobile phase" which is caused to travel through this column. **CLASSIFICATION OF CHROMATOGRAPHY METHODS**

1. **Partition chromatography:** In, this, the moving phase is the liquid while the stationary phase is a liquid film.
2. **Adsorption chromatography:** the moving phase here is a liquid while the stationary phase is solid.
3. **Gas-liquid chromatography:** the moving phase is gas and the stationary phase is a liquid film.
4. **Gas-solid chromatography:** The moving phase is gas and the stationary phase is a solid surface.

Stationary phase is solid with either acid or base functional group. The acid functional group can be carboxylic or sulphuric, while the base functional group could be amine or quaternary amine hydroxide.

Main chromatography methods are:

(i) High performance liquid chromatography
(ii) Thin layer chromatography
(iii) Paper chromatography
(iv) Ion-exchange chromatography.

TERMS USED IN CHROMATOGRAPHY

1. **ADSORBENT:** This is a solid material which serves as a stationary phase in adsorption chromatography.
2. **SUPPORT:** This is a support for the liquid film in partition chromatography.
3. **DEVELOPMENT:** this is a process of the flow of moving phase over the adsorbent or support.
4. **ELUTION:** This is the substance on the chromatography which is washed off the adsorbent.