A. GOOD LABORATORY PRACTICES (GLP)

There are some fundamentals of laboratory work such as handling of samples and how to behave in the laboratory. It is well known that laboratories are not the most healthy work environment. The presence of volatile hazardous organic liquids, explosive chemicals and laboratory equipment do not guarantee optimal conditions for a safe and healthy working place. Nevertheless, it is possible to organize the laboratory environment in such a way, that normal work is possible without health problems. To manage safe, healthy and trouble free environment in the laboratory some common rules should be followed by the analysts and attendants in the laboratories. These are known as Good Laboratory Practices (GLP) as given below.

A1. Safety Aspects

- All laboratory staff should be well versed and responsible for proper functioning of all the safety equipments and personal protective wares.
- Do not keep more chemicals in the laboratory than necessary for the ongoing work. Store the rest in a safe place.
- Always wear safety shoes, laboratory coats and safety glasses. Eating, drinking and smoking should be prohibited in the laboratory.
- Ensure that safety devices and precaution manuals are easy to find.
- Take care of fire extinguishers, fume hoods, chemical spill kit, eye washes and other safety devices. Always have a first-aid kit ready in the laboratory.
- Used liquids/chemicals should be disposed off in a proper way. Follow the procedure precisely.
- Don't work alone in the laboratory.
- Check analytical procedures if they are not clear, especially while working with organic liquids which may be dangerous.
- Be careful with power supply, gas cylinders and heating equipments.
- Work as much as possible in a fume hood and always add acid/base to water.
- Don't try to catch falling glassware. Remove and replace broken glassware.
- Instruct new laboratory persons in detail.
- Laboratory staff should undergo an annual medical examination.
- Safety procedures should be displayed as poster in the laboratory.
- Some training for laboratory staff is necessary for handling emergiencies.

A2. Laboratory Hazards

- Contact with chemicals may cause external or internal injuries. External injuries are caused by skin exposure to caustic/corrosive chemicals (acid/base/reactive salts). Prevent as far as possible inadvertent spills and splashes and equipment corrosion.

Internal injuries may result from toxic or corrosive effects of chemicals accidently ingested and absorbed by body.

- Inorganic acids and bases have health and safety limits. Exposure to fumes can irritate or damage eye, skin and create respiratory problems. Hot acids quickly react with the skin.
- Store acids and bases separately, in well-ventilated areas and away from volatile organic and oxidisable substances.
- Slowly add strong acids and bases to water to avoid spattering. If there is an accidental skin contact, thoroughly flush the contaminated area with water and seek medical attention.
- Perchloric acid reacts violently or explosively on contact with organic materials. Don't use perchloric acid together with organic reagents, particularly volatile solvents in one fume hood.
- NaOH and certain other chemicals produce considerable heat on dissolution, which may cause burns.
- Some metals (arsenic, nickel, mercury) are highly toxic and may also be carcinogenic. Avoid inhalation, ingestion and skin contact.
- Nearly all organic solvents are hazardous. Some are probably carcinogenic and should be treated with extra caution.
- Avoid mouth pipetting. Use of vacupads or auto pipettes is advisable.
- Beware of physical hazards from electrical items and gas cylinders.
- Never use glassware for HF (Hydrofluoric acid) treatment.
- Always remember, **HOT** glassware looks exactly like **COLD** glassware, be careful while handling.

A3. Preparing for the lab

The student - laboratory plays an important role in giving the student a "hands on" opportunity to verify chemical principles and learn important techniques for safe chemical manipulation. In order to get the most out of the laboratory work, simple suggestions have been enlisted below.

- Carefully read the laboratory experiment and any suggested additional reading (s) ***before*** coming to lab.
- Do the assigned pre-lab exercises (if any). These generally cover any calculations or important observations which need to be made.
- Make a list of questions regarding the experiment and get it clarified. This can save hours of wasted time in the laboratory.
- All data should be recorded in an appropriately bound log book. Do not use loose sheets or ring binders, as the loose sheets can be easily lost.

- Make a brief outline of the experiment including calculations for needed reagents/solutions in your notebook for a quick start.
- Laboratory procedures should be followed exactly as they are given in the literature. Any modification found necessary shall be tested through trials, published in a reputed journal first before adapting it for analysis.
- Prepare data recording format ahead of time. Well prepared data recording format not only speeds up the recording of data, but also helps greatly during calculations and report writing.
- Clean your glassware at the end of the lab period so that it is ready for the next laboratory work.

Many students *forget* to write down their observations. Color changes, endothermic or exothermic changes, changes in physical state, boiling point, melting point, freezing point, etc are very crucial to record. Look at the data, do they look reasonable for the type of experiment and expected results? When in doubt, repeat a portion of the experiment, there is no better lesson than to find your own mistake. If you are still unsure, ask the lab instructor.

Lab instructor will sometime discuss the important aspects of the lab with students individually or in small groups in an effort to help them get more out of the experiment.

Lastly, it is important to follow the **safety** do's and don'ts of the laboratory, not only for your own safety but also for your classmates. Report any dangerous lab practices of others; you will be doing them (and yourself) a big favour.

A4. Laboratory Tips for Students using Organic Substances

Organic Lab is the one lab where water can be the death of a reaction. Many students make the mistake of beginning each lab by washing their glassware. Soon, they find out, too much time has been wasted, their glassware is wet and they have no way to dry it. Clean your glassware at the end of a lab period so that it has time to dry for the next lab period. In case washing with water is inevitable, dry it with the help of acetone.

Always label reagents and flask contents. Many organic liquids are clear and colorless just like water, so are many aqueous solutions such as acids and bases.

Liquid organic reagents are best measured by volume. Most common organic liquids have their densities reported in one or more of the reference books (Mass/volume=density).

Speaking of liquids - Some fabrics (nylons, rayon etc.) dissolve when in contact with solvents such as acetone, ethyl acetate. Sulfuric acid (same acid used in car batteries) will leave holes in cotton clothing, even in dilute form. Don't wear your best clothes to lab and always use lab coats.

Some excellent sources for physical data on common organic chemicals encountered in the undergraduate laboratory (boiling point, melting point and density) are:

1) The CRC Handbook of Chemistry and Physics

2) Aldrich Chemical Company Catalog

3) The Merck Index

Cooling water always enters the bottom of the condenser and flows out the top at a gentle pace, no need to blow off hoses and soak you lab mates. Check to see if the water is running before heating starts.

Check the glass surface for its temperature before disassembling distillation and reflux equipment.

Many organics don't mix or dissolve in water and thus require special waste containers. Do not pour organics down the sink.

When performing extraction or separation, SAVE BOTH LAYERS (organic and water) until you are sure which contains the desired product. Can't remember which layer is which? Add a few drops of water to both from your wash bottle, the rest will be obvious.

Always stop distillation well before the boiling flask goes dry. Residues concentrated to dryness or near dryness during distillation may be unstable and explode. (This is particularly important with ethers and some alcohols which can form organic peroxides)

Use enough grease on glass joints to prevent "freezing" (but not so much that it drips from the joints) except for Teflon stopcocks, which are never greased. Glass joints which come in contact with "Strong Bases" (KOH, NaOH etc.) need to be essentially greased. Failure to clean them promptly after use will result in permanent sealing of the glass surfaces.

Never leave a reaction unattended. If you need to leave the lab for a few min. (restroom break, etc.) ask one of your classmates or the instructor to watch your reaction.

A5. Safety and Quality contract:

Any student, who wants to work in the laboratory, should sign a contract where he/she confirms that he/she has understood the contract which shall be in the following forms:

1. I will always follow instructions and guidelines given by the technical staff.
2. I will make the appropriate records to fulfil the demands of the log-book, including any quality control parameters mentioned in the log-book.
3. I will clean fume hoods, lab space, glass wares and equipment used at the end of each lab session.
4. I will identify and use the correct method of disposal for different types of waste and make sure that the waste receptacles are properly labelled.
5. I will follow good laboratory practice, including returning materials to their correct locations, proper care and handling of equipment.
6. I will always use a lab coat inside the laboratory.
7. I will use protective glasses when I am working with acids, bases and other corrosive chemicals.
8. I will plan and finish my work in the laboratory within normal working hours 9:00 – 17:30 (Monday-Friday). I will have a valid pass signed by my supervisor and laboratory in-charge if I have to work in the laboratory outside normal working hours and will follow the instructions given on my pass.
9. I will report any injury or a mishap immediately to my instructor and the person responsible for the room.
10. I will ensure closing of taps and switching off power points at the end of my work.

B. SOIL ANALYSIS

B1. Sampling and Processing

Sampling is an important step of any analysis. For cereals, vegetables, grasses and herbs the samples should be drawn from 0-15 cm depth. For plantation crops, trees and shrubs prepare composite sample from soil collected at depth of 0-30, 30-60 and 60-100 cm from 4 to 5 pits in about 0.5 ha field. The field sample is spread out on a tray for air drying. When it dries, sieve over a 2 mm sieve and store in an air tight polythene beg/glass / PVC/ porcelain jar. The sampling strategy may vary depending upon the shape of the land and intended purpose.

B2. Moisture

The results of soil analysis are to be calculated on the basis of oven dried sample weight. Therefore, the moisture analysis is executed before any other analysis. The result on the basis of the air-dry weight is multiplied by a moisture correction factor (mcf).

Procedure

- Place a porcelain crucible in the oven at a temperature of 105^0 C and leave it for 2 hours.
- Then cool it down to room temperature in a desiccator. Weigh the empty crucible (Empty Crucible weight =A).
- Weigh at least 10g of sample in the crucible and weigh it again (Sample + Crucible weight =B).
- Place the crucible at least 12 hours in the oven at 105^0 C. Then cool it down to room temperature in a desiccator and weigh again (C).

Calculation

$$\text{M (moisture content) \%} = \frac{(B-C)\times 100\%}{(C-A)}$$

$$\text{mcf (moisture correction factor)} = \frac{100+M(\%)}{100}$$

Reference

Buurman P., B. Van Langer and E.J. Velthrost, 1996. Manual for soil and water analysis. Backhuys Publishers, Leiden, The Netherlands.

Dhyan Singh, P.K.Chhonkar, R.N. Pandey, 1999. Soil, plant & water analysis - A method manual. IARI, New Delhi.

B3. Soil Texture

The particle size distribution of a soil expresses the proportions of the various size classes (clay < 0.002 mm, silt 0.002-0.02 mm and sand 0.02-2.0 mm particle size), commonly represented by weight percentages of the total soil. The proportions of these fractions are determined by Hydrometer method (Bouyoucos 1962) based on the Stokes`s Law which states that the rate of fall of particles in a suspension is directly proportional to their size.

Apparatus

Hydrometer, Mechanical stirrer, Thermometer, Suspension cylinders with 34 ± 2 cm height up to 1000 ml mark.

Reagents

- *Hydrogen peroxide H_2O_2 (30%)*.
- *Sodium hexametaphosphate 10%:* Dissolve 100g of sodium hexametaphosphate in distilled water and make it up to 1L solution.

Procedure

- Take 100 g of soil sample in a 1000 ml plastic beaker. Give H_2O_2 treatment to destroy the organic matter. Add 75 ml of distilled water and 15 ml of H_2O_2 and gently stir with a glass rod. If excessive frothing occurs, cool and add additional H_2O_2 till the reaction subsides (indicates complete destruction of organic matter). Skip this step if sample has extremely low organic matter.
- Add 200 ml distilled water, 100 ml Sodium hexametaphosphate solution, stir well with the help of a glass rod, cover and keep for 4 to 5 hrs.
- Make up to 500 ml volume and stir for 10 minutes. Transfer the whole content to a suspension cylinder making up to 1000 ml with distilled water.
- Close the cylinder with stopper tightly and shake several times to allow the soil particles to disperse completely.
- Remove the stopper and bring back adhering liquid on the stopper by touching it to the side of the cylinder. Immediately place hydrometer in the suspension. Record the reading exactly 40 second after placement of hydrometer (S_1 & B_1).
- Put back the stopper to close and invert the cylinder several times again to ensure complete dispersal of particles.
- Place the hydrometer into suspension exactly after 2 hrs and note the reading (S_2 & B_2).
- Simultaneously run a blank without soil and record the room temperature in ^{0}F.
- To stop foaming, add 2-3 drops of amyl alcohol.

Calculation

Correction factor (CF) = (Room temp in ^{0}F – 68) x 0.2

$$\text{Percent Silt + Clay} = \frac{(S_1\text{-}B_1) + CF}{\text{wt. of sample (g)}} \times 100$$

Where, S_1 and B_1 stand for hydrometer readings of sample and blank, taken at 40 seconds.

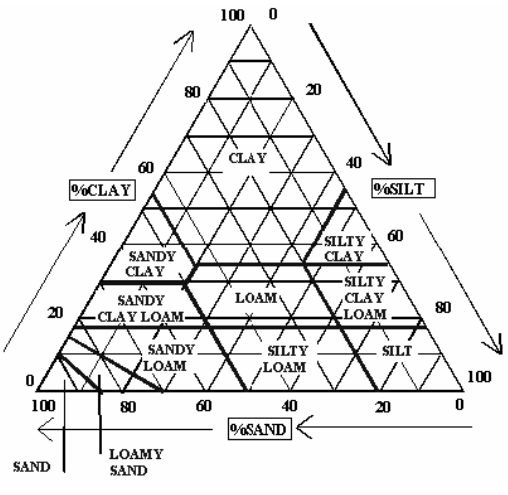
$$\text{Percent Clay} = \frac{(S_2\text{-}B_2) + CF}{\text{wt. of sample (g)}} \times 100$$

Where, S_2 and B_2 stand for hydrometer readings of sample and blank, taken after 2 hrs.

Percent Sand = 100 – (Silt + Clay)

Determination of texture

After determining the % of each constituent, plot these percentages on a nomograph. This will result in three lines being drawn, one from each side of the triangle according to the relative percentages. The point where these three lines meet will determine the description of the soil texture classes as shown in the diagram below:-



Reference

Bouyoucos G.J. 1962. Hydrometer method improved for making particle size analysis of soils. Agron. J.54: 464.

Dhyan Singh, P.K.Chhonkar, R.N. Pandey, 1999. Soil, plant & water analysis - A method manual. IARI, New Delhi.

B4. pH

pH of the soil is measured potentiometrically in a 1:2 or 1:5 soil - water suspensions or in saturates soil paste.

Apparatus

pH meter, mechanical shaker, extraction pump, Buchner funnel.

Reagents

- *Potassium chloride (KCl):* Dissolve 74.5 g of KCl in distilled water and make up to one litre to get 1M solution.
- *Buffer pH = 4, 7 and 9:* Dilute the respective ampoules according to the manufacturer's instructions.

Procedure

- Weigh 25 g of air dried, 2 mm sieved sample into a 100 ml flask and add 50 ml distilled water and shake for one hour.
- Calibrate pH meter using pH buffer and after that measure pH of suspension.

Reference

Dhyan Singh, P.K.Chhonkar, R.N. Pandey, 1999. Soil, plant & water analysis – A method manual. IARI, New Delhi.

USSL. 1954. Diagnostic and improvement of saline and alkali soils. USDA Handbook 60.

B5. Electrical Conductivity

The measurement of EC will give the concentration of soluble salts in the soil at any particular temperature. EC measured in 1:2 or 1:5 soil-water suspension with the help of conductivity meter.

Procedure

Calibrate EC meter using standard KCl solution and determine the EC of suspension used in pH determination.

B6. Soluble Anions and Cations (CO_3, HCO_3, Cl, SO_4, Ca, Mg, Na and K)

Procedure

- Prepare 1:2 or 1:5 soil-water suspension, shake for one hour on a shaker and filter through Whatman #1 filter paper and use the supernatant for analysis & extract of saturated soil paste.
- If supernatant is coloured then add activated charcoal and shake for 30 minute and filter the solution.
- Follow methods as given for water analysis.

B7. Organic Carbon (Walkely and Black 1934)

The organic carbon in the sample is oxidized with potassium dichromate and sulphuric acid. The excess potassium dichromate is titrated against ferrous ammonium sulphate.

Reagents

- *1N Potassium dichromate:* Dissolve 49.04 g $K_2Cr_2O_7$ in approx 500 ml distilled water and make up the volume to one litre.
- *Concentrated Sulphuric acid (H_2SO_4)*.
- *Concentrated Orthophosphoric acid (H_3PO_4).*
- *0.5N Ferrous ammonium sulphate:* Dissolve 196 g Ferrous ammonium sulphate in distilled water, add 20 ml of conc. H_2SO_4 and make volume up to one litre.
- *Diphenylamine indicator:* Dissolve 0.5 g of diphenylamine in a mixture of 20 ml distilled water and 80 ml conc. H_2SO_4.

Procedure

- Weigh 1 g soil into a 500 ml conical flask (Borosil/corning).
- Add 10 ml of 1 N $K_2Cr_2O_7$ and 20 ml of conc. H_2SO_4.
- Swril the flask carefully and allow it to stand for 30 minutes.
- Slowly add 200 ml distilled water and 10 ml H_3PO_4.
- Add 1 ml of diphenylamine indicator and titrate against 0.5 N Ferrous ammonium sulphate solution until green colour starts appearing indicating the end point.
- Run a blank simultaneously.

Calculation

$$\text{Organic Carbon (\%)} = \frac{10(B\text{-}S) \times 0.39 \times mcf}{B \times W}$$

Where

B = ml of ferrous ammonium sulphate solution used for blank.
S = ml of ferrous ammonium sulphate solution used for sample.
mcf = moisture correction factor.
W = sample weight (g).
0.39 = conversion factor (including a correction factor for a supposed 70% oxidation of organic carbon.

Note: High chloride content, as in case of saline soils, interferes in the estimation. It can be prevented by adding Ag_2SO_4 @ 1.25% to the conc. H_2SO_4.

References

Walkely A.J. and I.A. Black. 1934. Estimation of soil organic carbon by the chromic acid titration method. Soil sci. 37:29-38.

Jackson, M.L. 1962. Soil chemical analysis. Prentice Hall of India Pvt. Ltd. New Delhi.

B8. Nitrogen

B8a. Total Nitrogen (Kjeldhal method)

Nitrogen in soil/sediments is mostly present in the organic form with small quantities of ammonium and nitrate. This method measures only organic and ammoniacal form, therefore nitrate is excluded. The sample is digested in a mixture of H_2SO_4, K_2SO_4 and selenium (Se) which converts all N into ammonium sulphate. The distillation of ammonia (librated after sodium hydroxide is add to ammonium sulphate), over boric acid and titrated against standard acid to determine nitrogen.

Apparatus

Digestion block, Nitrogen distillation unit.

Reagents

- *Conc. H_2SO_4.*
- *Digestion catalyst:* Mixture of K_2SO_4/ Na_2SO_4 and Se (5 g:5 mg respectively).

- *40 % Sodium hydroxide:* Dissolve 400 g of NaOH in distilled water and make volume up to one litre.
- *2% Boric acid:* Dissolve 20 g of H_3BO_3 power in warm distilled water and dilute to one litre.
- *Mixed indicator:* Dissolve 70 mg of methyl red and 100 mg of bromocresol green in 100 ml of ethyl alcohol. Add 10 ml of this mixed indicator to each litre of 2% boric acid solution and adjust the pH to 4.5 with dil. HCl or dil. NaOH.
- *0.01N Sulphuric acid:* Prepare approximately 0.1N H_2SO_4 by adding 2.8 ml of conc. H_2SO_4 to about 990 ml of distilled water. Standardize it against 0.1 N standard NaOH solution. Dilute 10 times this 0.1N H_2SO_4 to get strength of 0.01 N.

Procedure

- Weigh 5 g of sample into digestion tube and moist it with distilled water.
- Add 20 ml of conc. H_2SO_4 and 5 g of catalyst and place the tube in digestion unit.
- Turn the heating equipment to about 400 ^{0}C and continue heating till the mixture is transparent and allow it to cool.
- Add 40% NaOH in digest till the colour change blackish and then distilled it.
- Collect the distillate (librated ammonia) into 10 ml of 2% boric acid solution.
- Titrate the distillate against 0.01N H_2SO_4 solution until pink colour starts appearing.
- Run a blank without soil for each set of samples.

Calculation

$$\text{Total N in soil (mg/kg)} = \frac{(\text{S-B}) \times \text{N} \times 14}{\text{Sample weight (g)}} \times 1000$$

Where
S= Volume of acid used against sample.
B= Volume of acid used against blank.
N= Normality of acid.

Reference

AOAC Official Methods of Analysis. Method 990.03. Protein(crude) in Animal Feed Combustion Method(Dumas method). 17th edition 2002. Reference: JAOAC 72, 770(1989).

Manual of Tecator digestion system 6/12. 1981.

B8b. Available Nitrogen (Subbiah and Asija 1956)

More than 90% of soil N exist as a complex organic compounds, which the plants cannot use directly. It becomes available to plants after its mineralization. The easily mineralisable N is estimated using alkaline $KMnO_4$, which oxidizes and hydrolyses the organic matter present in the soil. The liberated ammonia is condensed and absorbed in boric acid, which is titrated against standard acid.

Apparatus

Nitrogen distillation unit.

Reagents

- *0.32% Potassium permanganate:* Dissolve 3.2 g of $KMnO_4$ in distilled water and make volume up to one litre.
- *2.5% Sodium hydroxide:* Dissolve 25 g of NaOH in distilled water and make volume up to one litre.
- *2% Boric acid:* Dissolve 20 g of H_3BO_3 powder in warm distilled water and dilute to one litre.
- *Mixed indicator:* Dissolve 70 mg of methyl red and 100 mg of bromocresol green in 100 ml of ethyl alcohol. Add 10 ml of this mixed indicator to each litre of 2% boric acid solution and adjust the pH to 4.5 with dil. HCl or dil. NaOH.
- *0.01N Sulphuric acid:* Prepare approximately 0.1N H_2SO_4 by adding 2.8 ml of conc. H_2SO_4 to about 990 ml of distilled water. Standardize it against 0.1 N standard NaOH solution. Dilute 10 times this 0.1N H_2SO_4 to get strength of 0.01 N.

Procedure

- Weigh 10 g of soil sample in 250 ml Kjeldhal tube.
- Add 50 ml of 0.32% $KMnO_4$ solution and add 50 ml of 2.5% NaOH solution and distill immediately.
- Collect the distillate in 10 ml of 2% boric acid solution.
- Titrate the distillate against 0.01 N H_2SO_4 solution until pink colour starts appearing.
- Run a blank without soil for each set of samples.

Calculation

$$\text{Available N in soil (mg/kg)} = \frac{(\text{S-B}) \times \text{N} \times 14}{\text{Sample weight (g)}} \times 1000$$

Where

S= Volume of acid used against sample.

B= Volume of acid used against blank.

N= Normality of acid.

Reference

Subbiah, B.V. and G.L Asija. 1956. A rapid procedure for determination of available nitrogen in soils. Curr.Sci. 25:259-60.

B8c. Ammoniacal nitrogen

Like metallication, ammonium ions are also held in exchangeable form in soils. The NH_4-N is usually extracted with 10% NaCl or KCl solution. The NH_4-N in KCl/NaCl extract may be determined by distillation.

Apparatus

Kjeldhal distillation apparatus.

Reagent

- *Sodium chloride 10%:* Dissolve 100 gm of NaCl per liter and adjust the pH to 2.5 with HCl.
- 2% Boric acid.
- N/100 H_2SO_4.
- 40% NaOH.
- MgO.

Procedure

- Weigh 10 g fresh soil in 100 ml conical flask add 20 ml NaCl solution and shake for 30 minutes.
- Transfer the material into kjeldhal tube and add 20 ml 40% NaOH and distill.
- Collect the distillate in 2% Boric acid.
- Titrate the distillate against N/100 H_2SO_4.

Note: This recovers 99% NH_4-N. We can directly distillate fresh soil (without adding NaCl) by adding 0.5 g MgO or 20 ml NaOH. This recovers 92-95% NH_4-N.

Calculation

$$NH_4\text{-N in soil (mg/kg)} = \frac{(S\text{-}B) \times N \times 14}{\text{Sample weight (g)}} \times 1000$$

S= Volume of acid used against sample.

B= Volume of acid used against blank.

N= Normality of acid.

B9. Calcium carbonate

Carbonate in the sample is dissolved in the excess of hydrochloric acid. The remainder of the acid is titrated against sodium hydroxide. This method is known as Piper method.

Reagents

- *1N Hydrochloric acid:* Add about 800 ml distilled water into 1000 ml volumetric flask and carefully add 86 ml HCl 37%, allow to cool and makeup to volme 1 litre.
- *0.25N sodium hydroxide:* Dilute an ampule of 0.25 N NaOH according to manufacturer's instruction or dissolve 10 g in water, making 1 liter volume.
- *0.1% Phenolphthalein indicator:* Dissolve 100 mg of phenolphthalein in 100 ml ethanol.

Procedure

- Weigh 5 g of sample into a 250 ml conical flask.
- Add 100 ml of 1M HCl using pipette and swil gently. Let it stand overnight.
- Shake for two hours and let the suspension settle and pipette 5 ml of the supernatant to 100 ml conical flask and add 10 ml of water.
- Add 2-3 drops of phenolphthalein indicator and titrate with 0.25 M NaOH till colour change transparent to purple.

Calculation

$$CaCO_3\,(\%) = \frac{(B\text{-}S) \times N \times 100}{\text{Sample weight (g)}} \times \text{mcf}$$

Where

B = ml NaOH used for blank.
S = ml NaOH used for sample.
N = Normality of NaOH.
mcf = moisture correction factor.
100 = conversion factor.

Reference

C.S. Piper, 1966. Soil and plant analysis. Hans publications, Bombay.

Buurman, P., B. Van Langer & E.J. Velthrost, 1996. Manual for soil and water analysis. Backhuys Publishers, Leiden, The Netherlands.

Dhyan Singh, P.K.Chhonkar, R.N. Pandey, 1999. Soil, plant & water analysis - A method manual. IARI, New Delhi.

Page, A.L., R.H. Miller and D.R. Kenny. 1982. Methods of Soil Analysis, Part-I &II. Amer.Soc. Agron.. Madison, Wisconsin, USA.

B10. Phosphorous

B10a. Total Phosphorous

Phosphorus in soil and sediments can be determined in the acid digest by Vanadomolybdophosphoric yellow colour method.

Ammonium molybdate reacts under acidic conditions to form a heteropoly acid and molybdophosphoric acid. In the presence of vanadium, yellow vanadomolybdophosphoric acid is formed. The intensity of the yellow colour is proportional to phosphate concentration. Concentration ranges for different wavelengths are:

P Range (mg/l)	Wavelength (nm)
1.0 – 5.0	400
2.0 – 10	420
4.0 – 18	470

Reagents

- *Solution A:* Dissolve 25 g of ammonium molybdate in 300 ml warm distilled water and cool it.
- *Solution B:* Dissolve 1.25 g of ammonium metavandate in 300 ml boiled distilled water. Cool and add 250 ml conc. HNO_3. Cool solution B and mix with solution A and make up to one litre.
- *Standard P solution:* Dissolve 0.2195 g of dried KH_2PO_4 in distilled water, acidify with 25 ml of 7N H_2SO_4 and make the volume up to one litre to get 50 mg/l P solution.

Procedure

- Place 10 ml of acid digests (see chapter B-13) of soil sample in a 50 ml volumetric flask, add 10 ml of the vanadate-molybdate reagent and dilute to 50 ml.
- Mix well and read the P concentration after 10 minutes using spectrophotometer at 420 nm.
- Take 0, 1, 2, 3, 4 and 5 ml of the 100 mg/l P solution in 50 ml volumetric flask and develop colour in identical manner.
- Calibrate the spectrophotometer with known P concentration and read the concentration of sample.

Calculation

$$P\ \mu g/g = \frac{R \times 50}{10} \times \frac{100}{\text{Sample wt. (g)}}$$

Where,

R = reading of spectrophotometer P mg/l.
10 = volume of acid digest used for colour development.
50 = Volume make up for colour development.
100 = Volume make up after acid digestion.
g = Sample wt. (g) for acid digestion.

B10b. Available Phosphorous

Two procedures for analyzing available phosphorus are “Bray’s” method for acid soils and the “Olsen’s” method for other soils.

P- BRAY [Bray & Kurtz, 1945]

Principle

Phosphate is extracted in an acidic ammonium fluoride solution. After the extraction, the phosphate is determined colorimetrically with ammonium molybdate as the colouring reagent. This method is used for acidic soils.

Apparatus

Spectrophotometer.

Reagents

- *Ammonium fluoride- 1 M (a):* Dissolve 3.7 g NH_4F in 100 ml distilled water and store it in a polythene bottle.
- *Hydrochloric acid-0.5 M (b):* Bring 80 ml water to a 100 ml measuring cylinder and add 4.3 ml HCl 37%. Allow to cool and make the volume 100 ml.
- *Extractant:* Take 460 ml water in a 500 ml measuring cylinder and add 15 ml of the solution (a) and 25 ml of solution (b) and store it.
- *Boric acid (1%):* Dissolve 1 g H_3BO_3 in 100 ml water.
- *Sulphuric acid (2.5 M):* Carefully add 35 ml H_2SO_4 96% to 200 ml water. Allow to cool and make it to 250 ml with water.
- *Ammonium molybdate (4%):* Dissolve 4 g ammonium molybdate in 100 ml water and store it in dark.

- *Potassium antimony tartrate (0.275%):* Dissolve 275 mg Potassium antimony tartrate in 100 ml water.
- *Ascorbic acid (1.75%):* Dissolve 1.75 g Ascorbic acid in 100 ml water. (prepare fresh daily).
- *Mixed Reagent:* Add, using a measuring cylinder, successively the following reagents to a 500 ml bottle; 50 ml 2.5 M Sulphuric acid, 15 ml 4% Ammonium molybdate, 30 ml 1.75% Ascorbic acid, 5 ml 0.275% Potassium antimony tartrate and 200 ml water. Mix well after each addition and prepare fresh reagent daily
- *Standard series of Phosphorus:* Weigh 4.3943 g KH_2PO_4 (dried at 105^0C) into a 250 ml beaker; add about 200 ml water and let dissolve. Make volume up to one liter to get 1000 ppm standard P solution.

Pipette 10 ml of the 1000 ppm stock solution in a 100 ml volumetric flask. Make up to volume with water and homogenize. This makes a 100 ppm P solution.

Pipette 30 ml of this 100 ppm P solution to a 250 ml volumetric flask. Make to volume with water and homogenize. This makes a 12 ppm P solution.

Take 0, 5, 10, 15, 20, and 25 ml of 12 ppm P solution to a 50 ml volumetric flask and make up to the volume with water to get a series of standards of; 0.0, 1.2, 2.4, 3.6, 4.8 and 6.0 ppm P.

Procedure

- Weigh 2 g of soil sample into a 50 ml shaking bottle, add 14 ml of extractant, shake for one minute by hand and filter immediately by using Whatman # 42 filter paper. The filtration may not exceed 10 minutes.
- Pipette successively in a test tube, 1 ml of the standard series, sample or blank, add 2 ml of boric acid and 3 ml of mixed reagent.
- Shake and wait for one hour for the blue colour development.
- Measure the concentration of the solution at 882 nm or 720 nm, using spectrophotometer.
- Initially standardize spectrophotometer with a series of solutions of known Phosphorus concentration.

Calculation

$$\text{Available P (mg/kg)} = \frac{(S\text{-}B) \times D \times [14 + \{W - (\frac{W}{mcf})\}]}{W\ (g)} \times mcf$$

Where

S = P concentration in sample (mg/l) read by spectrophotometer

B = P concentration in blank (mg/l) read by spectrophotometer.

D = Dilution factor (standard 1 for undiluted samples).

W = Sample weight

mcf = moisture correction factor.

14 = volume of extractant.

Reference

Bray, R.H. & L.T. Kurtz. 1945. Determination of total, organic and available form of Phosphorous in soils. Soil Sci. 59:39-45.

Buurman P., B. Van Langer and E.J. Velthrost, 1996. Manual for soil and water analysis. Backhuys Publishers, Leiden, The Netherlands.

Dhyan Singh, P.K.Chhonkar, R.N. Pandey, 1999. Soil, plant & water analysis - A method manual. IARI, New Delhi.

P- OLSEN [Olsen et al, 1954]

Principle

Phosphate in the soil sample is extracted using sodium bicarbonate solution of pH=8.5.in an acid ammonium fluoride solution. After the extraction, the phosphate is determined colorimetrically with ammonium molybdate as the colouring reagent. This method is used for alkaline, calcareous or neutral soils.

Apparatus

Spectrophotometer.

Reagents

- *Sodium hydroxide (1M):* Dissolve 4 g NaOH in 100 ml distilled water.
- *Sodium bicarbonate (0.5 M):* Dissolve 42 g $NaHCO_3$ in one litre water and adjust pH to 8.5 with 1M NaOH (check pH every day).
- *Sulphuric acid (4M):* Carefully add 56 ml conc. H_2SO_4 to 150 ml water and allow to cool and make up to 250 ml with water.
- *Ammonium molybdate (4%):* Dissolve 4 g Ammonium molybdate in 100 ml water and store it in a polythene bottle in dark.
- *Potassium antimony tartrate (0.275%):* Dissolve 275 mg Potassium antimony tartrate in 100 ml water.
- *Ascorbic acid (1.75%):* Dissolve 1.75 g Ascorbic acid in 100 ml water. (Prepare fresh daily).
- *Mixed Reagent:* Add successively, using a measuring cylinder, the following reagents to a 500 ml bottle; 50 ml 4M Sulphuric acid,15 ml 4% Ammonium molybdate, 30 ml 1.75% Ascorbic acid, 5 ml 0.275% Potassium antimony tartrate, 200 ml water. Mix well after each addition and prepare fresh daily.
- *Standard series of Phosphorus:* Weigh 4.3943 g KH_2PO_4 (dried at 105^0C) into a 250 ml beaker, add about 200 ml water and let dissolve. Make volume up to one liter to get 1000 ppm standard P solution.

Pipette 10 ml of the 1000 ppm stock solution in a 100 ml volumetric flask. Make up to volume with water and homogenize. This makes a 100 ppm P solution.

Pipette 10 ml of this 100 ppm P solution to a 250 ml volumetric flask. Make up to volume with extracting solution and homogenize. This makes a 4 ppm P solution. Pipette out of this 4 ppm P solution respectively 0, 5, 10, 15, 20, and 25 ml in to a 50 ml

volumetric flask and make each flask up to volume with extracting solution to get a series of standards of; 0.0, 0.4, 0.8, 1.2, 1.6 and 2.0 ppm P.

Procedure

- Weigh 5 g of sample into a 250 ml shaking bottle, add 100 ml of sodium bicarbonate extractant, shake for 30 minutes and filter through Whatman # 42 filter paper. Pipette 5 ml of the standard series, sample or blank to a test tube and add 5 ml of mixed reagent.
- Shake and stand for one hour for the blue colour development.
- Measure the concentration of the solution at 882 nm or 720 nm, using spectrophotometer.
- Initially standardize spectrophotometer with a series of known concentration after that determine phosphorus concentration in the sample.

Calculation

$$\text{Available P (mg/kg)} = \frac{(S\text{-}B) \times D \times [100 + \{W - (\frac{W}{mcf})\}]}{W\ (g)} \times mcf$$

Where

S = P concentration in sample (mg/l) read by spectrophotometer.

B = P concentration in blank (mg/l) read by spectrophotometer.

D = Dilution factor (standard 1 for undiluted samples).

W = Weight of sample.

mcf = Moisture correction factor.

100 = Volume of extractant.

Reference

Olsen, S.R., C.V. Cole, F.S. Watanabe and L.A. Dean. 1954. Estimation of available phosphorus in soils by extraction with sodium bicarbonate. Cire USQep. Agri. Q 39.

Buurman P., B. Van Langer and E.J. Velthrost. 1996. Manual for soil and water analysis. Backhuys Publishers, Leiden, The Netherlands.

Dhyan Singh, P.K.Chhonkar, R.N. Pandey. 1999. Soil, plant & water analysis – A method manual. IARI, New Delhi

B11. Available potassium

Potassium in soil exists as water soluble, exchangeable, fixed (lattice-K). The first two forms constitute only small part (not more than 1%) and are considered to be easily available to plant. These forms are determined by Ammonium acetate method of Hanway and Heidel (1952).

Apparatus

Flame photometer, Shaker, pH meter.

Reagents

- *Ammonium acetate (1N):* Dissolve 77.08 g of ammonium acetate in 500 ml of distilled water and makeup volume to one litre. Adjust pH to 7.0 with glacial acetic acid or ammonia solution.
- *Standard K solutions:* Dissolve 1.908 g oven dried KCl salt in one litre distilled water to get 1000 ppm K solution. Dilute this solution to get solutions of 10, 25, 50, 75 and 100 ppm K in ammonium acetate working solution.

Procedure

- Weigh 5 g of soil sample in 100 ml conical flask.
- Add 25 ml 1N ammonium acetate solution and shake for 5 minutes.
- Filter through Whatman #1 filter paper and measure K concentration in the filtrate using flame photometer.

Calculation

$$\text{Available K (mg/kg)} = \frac{C \times 25}{\text{Sample weight (g)}} \times \text{mcf}$$

Where

C = Concentration of potassium in filtrate.

mcf = Moisture correction factor.

25 = Volume of Ammonium acetate.

Reference

Hanway, J.J. and H. Heidel. 1952. Soil analysis methods as used in Iowa state college soil testing laboratory. Iowa Agri. 57:1-31.

Jackson, M.L. 1962. Soil chemical analysis. Prentice Hall of India Pvt. Ltd. New Delhi.

Page, A.L., R.H. Miller and D.R. Kenny. 1982. Methods of Soil Analysis, Part-I &II. Amer.Soc. Agron.. Madison, Wisconsin, USA.

Note: Same filtrate can be used for determination of available sodium.

B12. Available micronutrients (DTPA Extractable)

Available micronutrient extracted from soil by DTPA according to Lindsay and Norvell (1978). DTPA is a chelating agent which combines with free metal ions in solution and forms soluble complexes. DTPA offer most favorable combination for the determination of Zn, Fe, Cu and Mn.

Reagent

- *Standards of Zn, Fe, Cu, Mn.*
- *DTPA extractant:* Dissolve 1.967 g of Diethyline-triamine-penta acetic acid (DTPA) and 1.470 g of $CaCl_2.2H_2O$ in about 25 ml of double distilled water (DDW) by adding 13.3 ml of Triethanolamine (TEA), followed by 100 ml of DDW. Make up to one litre volume, adjusting pH to 7.3 with dilute HCl.

Procedure

- Weigh 10 g of soil in 100 ml conical flask.
- Add 20 ml of DTPA extractant and shake for 2 hrs on a mechanical shaker.
- Filter through Whatman # 42 filter paper and measure the Zn, Fe, Cu and Mn in filtrate by Atomic Absorption Spectrophotometer (AAS).

Calculation

$$\text{Element in soil (mg/kg)} = \frac{C \times 20}{\text{Sample weight (g)}} \times \text{mcf}$$

Where
C = Concentration of element in filtrate.
mcf = Moisture correction factor.
20 = Volume of DTPA extractant.

Reference

Lindsay, W.L. and Norvell, W.A., 1978. Development of a DTPA soil test for zinc, iron, manganese and copper. Soil Sci. Soc. Am. J. 42:421-448.

B13. Sample Preparation for Elemental Analysis

For the release of mineral elements from soil and sediments, wet oxidation of sample is carried out. Wet oxidation employs oxidizing acids like HNO_3- $HClO_4$-HF tri-acid mixture or HNO_3-$HClO_4$ di-acid mixture. Use of $HClO_4$ avoids the volatilization loss of K and provides a clear solution while HF helps removing silica. The di-acid oxidation method is easier, less time-consuming and convenient but it is not a total digestion as soil does not dissolve completely, particularly silicate minerals, therefore, di-acid digestion is known as pseudo digestion or partial digestion.

B13a. HNO_3/$HClO_4$ Digestion

- Weigh 0.5-1.0 g sample of air dried soil in digestion tube and add 3 ml conc. HNO_3 digest on electrically heated block for 1 h at 145^o C.
- Then add 4 ml of $HClO_4$ and heat it to 240^o C for to further one hour.
- Cool and filter through Whatman # 42 filter paper and makeup to 50 ml volume.
- Determine P, K, Na, Ca, Mg, Al, B, Fe, Mn, Cu, Zn, Cd, Ca, Ni, Pb, Co, Mo, Si, Ti, Sr, V, and Ba by ICP-AES or ICP-MS or AAS.

B13b. Microwave Digestion

- Weigh 0.5g sample of air dried soil in MF/HF vessels of microwave. Add 5 ml conc. HNO_3 and 1 ml of HCl. Insert the vessels into the rotor of microwave.
- Set the microwave system as; Temp.100^o C/ vessel, Ramp-10 minute, Hold- 10 minute and cooling 30 minute.
- After cooling, filter through Whatman # 42 filter paper and makeup to 50 ml volume.
- Determine P, K, Na, Ca, Mg, Al, B, Fe, Mn, Cu, Zn, Cd, Ca, Ni, Pb, Co, Mo, Si, Ti, Sr, V, and Ba by ICP-AES or ICP-MS or AAS.

B13c. $HCl0_4$-HF Digestion

- Weigh 0.5-1.0 g of sample into a clean 100 ml teflon beaker, wet with 5 ml of distilled water.
- Add two ml of $HCl0_4$ (70%) and 12 ml of HF (40%), and heat to incipient (near dryness).
- Again add 8 ml of HF and heat to dryness.
- Now add two ml of $HCl0_4$ and about 5 ml of distilled water and heat to incipient.
- Dissolve the remaining residue in 8 ml of HCl and 20 ml of water.
- Makeup to the 100 ml volume and store in polyethylene bottle.
- Determine P, K, Na, Ca, Mg, Al, B, Fe, Mn, Cu, Zn, Cd, Ca, Ni, Pb, Co, Mo, S, As, Ti, Sr, V, and Ba in the supernatant by ICP-AES or AAS or ICP-MS.

References

Dahlquist, R.L. and J.W. Knoll. 1978. Inductively Coupled Plasma-Atomic Emission Spectrometry: Analysis of biological materials and soils for major trace, and ultra-trace elements. Appl. Spectroscopy 32:1-30. ICP: ARL (Fisons) Model 3560 ICP-AES.

Blancher, R.W., G. Rehm, and A. C. Caldwell. 1965. Sulfur in plant materials by digestion with nitric and Perchloric acid. Soil Sci. Soc. Am. Proc. 29:71-72.

EPA Method No. 3051. 1992. Microwave assisted acid digestion of sediments, sludges, soils, and oils. In Test Methods of Evaluating Solid Waste, Physical/Chemical Methods, SW-846, 3rd Edition. U.S. Environmental Protection Agency

Jackson, M.L. 1958. Soil Chemical Analysis. Prentice-Hall, Englewood Cliffs, NJ, USA.

Hossner, L.R. 1996. Dissolution for total elemental analysis. In: Methods of Soil Analysis, Part 3—Chemical Methods. Soil Science Society of America, Madison, WI, USA.

C. WATER ANALYSIS

C1. Sampling and processing

The most important issue when drawing up a plan for sampling is to clearly understand its significance and objectives. In other words, we must correctly determine how and why to conduct the survey for collection of samples. Furthermore, sampling work should be undertaken according to specified procedures. The samples can be of two types i.e. grab or composite.

Grab samples are single samples collected at a specific spot at a site over a short period of time. Thus they represent only the composition of its source at the time and place of collection.

Composite samples are obtained by taking an appropriate number of grab samples collected at equal intervals or proportional to flow. Flow proportional composite samples are collected when the flow and waste characteristics are continually changing.

An appropriate sampling can be made by using sampling tools. Manual sampling tools include dippers, 'bacon bombs', weighted bottles and hand operated pumps. A dipper consists of a wide-mouthed corrosion resistant container on long handle that is used to dip the sample from the bulk material and then pour it into the sample container for transfer to the laboratory. The dipper container should be constructed of stainless steel or Teflon. Weighted bottles are sample collection devices which are attached to a pole and have a heavy hinged stopper with a string attached. A similar device is called a "bacon bomb". The bottle is lowered to the appropriate depth with the stopper in place, then the stopper is pulled open with the string, filling the container with the sample at the desired depth. After the bottle is filled, the stopper is allowed to settle back in to place, the closed bottle is raised to the surface and the sample is brought to the laboratory.

Electrical, mechanical, and pneumatic samplers are available. Great care must be taken in the selection of the proper instrument for sampling based on knowledge of the operating conditions within the plant and the nature of the waste water being sampled.

Normally, polyethylene or colorless glass bottles with ground stoppers are used as sample containers. Samples should not become contaminated and the components targeted for testing should not be allowed to escape from the sample in the containers, so it must be made of a quality material and it should be possible to close it securely. They should only be used after being sufficiently rinsed to avoid any impediment to testing. Rubber and cork stoppers must not be used to avoid the risk of contaminating the sample. Prior to usage, rinse bottles first with tap water and then with distilled or ion

exchange water. When testing for trace amounts of metals, rinse them with warmed nitric acid (1+10) or warmed hydrochloric acid (1+5), then with tap water again, and finally rinse them out with distilled or ion exchange water. When used bottles are re-used, rinse them with warmed nitric acid (1+10), warmed hydrochloric acid (1+5), or medical rinsing liquid such as a surface-active agent, rinse them again with tap water, and finally rinse them out with distilled or ion exchange water. When some matter is adhered, use a brush with cleanser or something suitable to give it a pre-wash before taking the above action. (Soaps are not suitable as they are liable to remain inside the bottle.) Cleansers that include components being targeted in the test should not be used.

Once the sample has been taken, record certain observations and measurements before sending it to the laboratory. These observations should include appearance (color, haziness, turbidity, presence of floc, oil on the surface), odor, and measurements that are valid only on a fresh sample (temperature, dissolved oxygen and pH).

After the sampling, preservation of samples is the main thing to obtain good quality result. By far, the most common preservative is **cooling to 4 °C**. This can be achieved by making slurry of ice and water and placing the collected sample in the slurry. This serves to slow biological activity in the sample and keep dissolved gases in solution. However, it can hasten certain physical processes such as precipitation of metals. Other common preservatives include, use of sodium thiosulfate at a final concentration of 0.008 % (80 mg/L of sample) to remove chlorine, addition of copper sulfate or mercuric chloride to halt biological activity, and addition of zinc acetate to trap sulfides. Appropriate pH adjustment to either, over 12 SU (**S**tandard **U**nits) with sodium hydroxide or less than 2 SU with hydrochloric, sulfuric or nitric acid (depending on the intended analyte) can inactivate biological processes, prevent known chemical reactions and maintain a target analyte in solution. The analyst must be aware that acidification of nitrate-nitrite samples with nitric acid **or** preservation of sulfate samples with sulfuric acid will invalidate the analyses, so the sampler must make adequate notation. Samples which are not preserved must be analyzed immediately. A summary for collection and preservation of samples as per APHA (1998) is given below in table 1.

Table.1. summary for collection and preservation of samples as per APHA (1998).

Determination	Container	Sample size (*ml*)	Preservation	Maximum storage
Acidity	P, G(B)	100	Refrigerate	24 h
Alkalinity	P, G	200	Refrigerate	24 h
BOD	P, G	1000	Refrigerate	6h
Boron	P	1000	$HN0_3$ to pH <2	28 d
Bromide	P, G	100	None required	28 d
Carbon, organic, total	G (B)	100	Analyze immediately; or refrigerate and add HCl, H_3PO_4, or H_2SO_4 to pH <2	7 d
Carbon dioxide	P, G	100	Analyze immediately	0.25 h
COD	P, G	100	Analyze as soon as possible, or add H_2SO_4 to pH <2; refrigerate	7d
Chloride	P, G	50	None required	N.S.
Chlorine, total, residual	P, G	500	Analyze immediately	0.25 h
Chlorine dioxide	P, G	500	Analyze immediately	0.25 h
Chlorophyll	P, G	500	Dark, 4°C	24-48 h
Color	P, G	500	Refrigerate	48 h
Conductance	P, G	500	Refrigerate	28 d
Cyanide	P, G	1000	Add NaOH to pH >12, refrigerate in dark	
Fluoride	P	100	None required	28 d
Hardness	P,G	400	Add HNO3 or H2SO4 to pH <2	6 months
Iodine	P, G	500	Analyze immediately	0.25 h
Metals, general	P(A), G(A)	1000	Filter immediately, add HNO_3 to pH <2	6 months
Chromium VI	P(A), G(A)	1000	Refrigerate	24 h
Mercury	P(A), G(A)	1000	Add HNO3 to pH <2, 4°C,	28 d
Ammonia	P, G	500	Analyze as soon as possible or add H2SO4 to pH <2, refrigerate	7 d
Nitrate	P, G	100	Analyze as soon as possible; refrigerate	48 h
Nitrate + nitrite	P, G	200	Add H_2SO_4 to pH <2, refrigerate	1-2 d
Nitrite	P, G	100	Analyze as soon as possible; refrigerate	none
Organic, Kjeldahl-N	P, G	500	Refrigerate, add H_2SO_4 to pH <2	7d
Odor	G	500	Analyze as soon as possible; refrigerate	6h
Oil and grease	G	1000	Add HC1 or H_2SO_4 to pH <2, refrigerate	28 d

Determination	Container	Sample Size (*ml*)	Preservation	Maximum Storage
MB As	P, G	250	Refrigerate	48 h
Pesticides*	G(S),	1000	Refrigerate, add 1000 mg ascorbic acid/L if residual chlorine present	7d
Phenols	P, G	500	Refrigerate, add H_2SO_4 to pH <2	*
Base/neutral&acids	G (S)	1000	Refrigerate	7d
Dissolved Oxygen	G, BOD	300	Analyze immediately	0.25h
Ozone	G	1000	Analyze immediately	0.25h
pH	P, G	50	Analyze immediately	0.25h
Phosphate	G (A)	100	Refrigerate	48h
Phosphorus total	P, G	100	Add H_2SO_4 to pH <2, Refrigerate	28d
Salinity	G	240	Use wax seal	6 month
Silica	P	200	Refrigerate	28d
Solids	P, G	200	Refrigerate	7d
Sulphate	P, G	100	Refrigerate	28d
Sulphide	P, G	100	Refrigerate; add 4 drops 2N zinc acetate/100ml; add NaOH to pH>9	28d
Temperature	P, G	-	Analyze immediately	0.25h
Turbidity	P, G	100	Refrigerate in dark	24h

*For determinations not listed, use glass or plastic containers; preferably refrigerate during storage and analyze as soon as possible.

P = plastic (polyethylene or equivalent); G = glass; G(A) or P(A) = rinsed with 1 + 1 HNO_3; G(B) = glass, borosilicate; G(S) = glass, rinsed with organic solvents or baked
Refrigerate = storage at 4°C ± 2°C; in the dark
N.S. = not stated in cited reference; stat = no storage allowed; analyze immediately.

References

APHA, 1998. Standard methods for the examination of waters and wastewaters. APHA-AWWA-WEF, Washington, DC.

C2. pH

The pH of water is measured with the help of a pH meter, using a glass combination electrode saturated with KCl.

Apparatus

pH meter.

Reagents

- *Potassium chloride (1M):* Dissolve 74.5 g of KCl in distilled water and makeup to one litre to get a 1M solution.
- *Buffer pH = 4, 7 and 9:* Dilute the respective ampoules according to the manufacturer's instructions.

Procedure

- Calibrate pH meter using pH buffer before measuring pH of the sample.
- Take a 50 ml of sample in 100 ml flask, dip the electrode and read the pH of the solution.

References

Buurman P., B. Van Langer and E.J. Velthrost, 1996. Manual for soil and water analysis. Backhuys Publishers, Leiden, The Netherlands.

Dhyan Singh, P.K.Chhonkar, R.N. Pandey, 1999. Soil, plant & water analysis - A method manual. IARI, New Delhi.

APHA, 1998. Standard methods for the examination of waters and wastewaters. APHA-AWWA-WEF, Washington, DC.

C3. Electrical Conductivity

The measurement of EC will give the concentration of soluble salts in the sample at any particular temperature. The EC is measured directly with the help of conductivity meter.

Procedure

Calibrate EC meter using standard KCl solution and determine the EC of sample in dS/m or m mho/cm. The conductivity meter can be standardized with the three standard KCl solutions. Conductivity of standard solutions at 25^0C are given below. Set the conductivity meter on desired KCl solution.

Concentration of KCl	Conductivity (dS/m)	Temp. coefficient at 25^0C
0.1M	12.88	+1.90%
0.01M	1.413	+1.94%
0.001M	0.146	+2.04%

The above table can be used to calculate the conductivity at any other ambient temperature by using following formula.

Cond. at D^0C = C 25^0C + (C25^0C x 1.94/100 x (D^0C - 25^0C).

Where C = conductivity, D= Desired temp.

Note: Conductivity cell of 0.1K should not be standardized with 0.1M KCl as the conductivity of this solution falls beyond its range.

References

Buurman P., B. Van Langer and E.J. Velthrost, 1996. Manual for soil and water analysis. Backhuys Publishers, Leiden, The Netherlands.

Dhyan Singh, P.K.Chhonkar, R.N. Pandey, 1999. Soil, plant & water analysis - A method manual. IARI, New Delhi.

APHA, 1998. Standard methods for the examination of waters and wastewaters. APHA-AWWA-WEF, Washington, DC.

C4. Soluble Anions and Cations

C4a. Carbonate and Bicarbonate (CO_3^{2-} & HCO_3^-)

Carbonates and bicarbonates in water can be determined by titrating a known volume of water against standard H_2SO_4 using phenolphthalein and methyl orange indicators respectively.

Reagents

- *Sulphuric acid 0.01N:* Add 2.8 ml of conc. H_2SO_4 into 500 ml distilled water and dilute to one litre to get approximately 0.1N H_2SO_4. Dilute it 10 times and standardize it by titrating against standard alkali.
- *Phenolphthalein indicator:* Dissolve 0.25 g of phenolphthalein powder in 100 ml of 60% ethanol.
- *Methyl orange indicator:* Dissolve 0.50 g of methyl orange in 100 ml of 95% ethanol.

Procedure

- Transfer 10 ml of sample to a 100 ml conical flask and add 2 drops of phenolphthalein indicator. Pink color shows the presence of carbonate, titrate against 0.01N H_2SO_4 until the sample is colour less. Denote the volume as 'A".
- Now add two drops of methyl orange indicator, yellow colour will appear. Continue titration with 0.01N H_2SO_4 until the yellow colour changes to red. Denote the volume as 'B".

Calculation

$$CO_3^{2-} \text{ me/l} = \frac{2 \times N \times A}{\text{ml of sample}} \times 1000$$

$$CO_3^{2-} \text{ mg/l} = \frac{2 \times N \times A}{\text{ml of sample}} \times 1000 \times 30$$

$$HCO_3^- \text{ me/l} = \frac{N \times (B - A)}{\text{ml of sample}} \times 1000$$

$$HCO_3^- \text{ mg/l} = \frac{N \times (B - A)}{\text{ml of sample}} \times 1000 \times 61$$

Where

N = Normality of acid.

2 = Valancy of carbonate.
A = Volume of titrant against phenolphthalein indicator.
B = Volume of titrant against methyl orange indicator.
30 = Equivalent weight of CO_3.
61 = Equivalent weight of HCO_3.

C4b. Chloride (Cl^-)

Chlorides being highly soluble are present in all waters but the amount is often very low in natural waters. The determination of chlorides is done by $AgNO_3$ (Mohrs's titration) method. Potassium chromate shows the brick red colour at the end point due to formation of silver chromate.

Reagents

- *Silver nitrate solution 0.01N:* Dissolve 1.7 g of $AgNO_3$ in distilled water and make volume to one litre.
- *Potassium chromate indicator:* Dissolve 5 g of K_2CrO_4 in 100 ml of distilled water.

Procedure

- Transfer 10 ml of sample to a 100 ml conical flask and add 2 drops of potassium chromate indicator.
- Yellow colour will appear, titrate against 0.01N $AgNO_3$ until colour changes to brick red.

Calculation

$$Cl^- \text{ me/l} = \frac{N \times V}{\text{ml of sample}} \times 1000$$

$$Cl^- \text{ mg/l} = \frac{N \times V}{\text{ml of sample}} \times 1000 \times 35.5$$

NaCl mg/l = Cl mg/l x 1.65

Where
N = Normality of $AgNO_3$.
V = Volume of $AgNO_3$ used in titration.
35.5 = Equivalent weight of Chloride.

C4c. Sulphate (SO_4^{2-})

Reagents

- *Standard Ca solution (0.02 N):* Dissolve 1.001 g of AR grade dried $CaCO_3$ in about 5 ml of dil. HCl (1:50), boil to expel the CO_2 and dilute to one litre with distilled water.
- *Eriochrome black T indicator:* Dissolve 0.5 g of EBT dye and 4.5 g of hydroxylamine – hydrochloride (AR grade) in 100 ml of ethyl alcohol.
- *Standard HCl (0.02 N):* Dilute appropriate volume of conc. HCl so as to get approximately 0.02N solution. Standardize it against standard alkali to exact normality.
- *Buffer solution:* Dissolve 8.25 g of NH_4Cl and 113 ml conc. Ammonia solution (NH_4OH) per liter, to get a pH of 10. 10 ml of this solution is added to 50 ml of water sample.
- *Standard $MgCl_2$ solution (0.02 N):* Dissolve 0.843 g of dried $MgCO_3$ in little excess of dilute HCl and dilute to one litre with distilled water. Standardize against 0.02 N EDTA solution.
- *Standard $BaCl_2$ solution (0.02 N):* Dissolve 2.4428 g of AR grade $BaCl_2.2H_2O$ in about 300 ml of distilled water and dilute to one litre. Standardize against 0.02 N Ca solution.
- *Standard EDTA solution (0.02 N):* dissolve 4.0 g of pure EDTA-disodium salt in about 200 ml of distilled water and dilute to one litre. Standardize against 0.02 N Ca solution.

Procedure

- Determine the Ca^{2+} + Mg^{2+} content of the sample by titrating 5 ml of sample.
- Determine CO_3^{2-} and HCO_3^- content by titrating against 0.02 N HCl just as with 0.01 H_2SO_4.
- To a third aliquot, add measured volume of 0.02 N HCl equivalent to total alkalinity (carbonates + bicarbonates) with a little excess and boil to destroy the carbonate and drive off CO_2.
- Add a known volume of standard $BaCl_2$ solution enough to exceed the sulphate content in aliquot and allow boiling for a few seconds.
- Cool the contents and add 10 ml of buffer and 5 drops of EBT indicator.
- Titrate against the standard EDTA solution to the first end point. This should not be taken as the final reading as the accuracy is poor even if the EDTA solution contains some Mg^{2+} ions.
- Add a known volume (*e.g.* 2 ml) of standard $MgCl_2$ solution and resume the titration as for Ca^{2+} + Mg^{2+} to get a similar end point.

Calculation

SO_4^{2-} me/L = B + Ba + Mg – T

Where,
B = Ca + Mg me/l in original sample.
Ba = me of Ba added.
Mg = me of Mg added.
T = me of EDTA in the total titration of the sample (including Ba and Mg).

C4d. Calcium and Magnesium (Ca^{2+}& Mg^{2+})

For the determination of both of these, the versenate titration method is used in which EDTA-disodium salt solution is used to chelate them. Calcium is separately estimated by versenate method using ammonium purpurate (Murexide) indicator and thus magnesium can be obtained by deduction of Ca from Ca+Mg content. Both the cations can also be estimated by AAS.

Reagents

- *Standard versenate solution (0.01N):* Dissolve 2.0 g of EDTA- disodium salt in distilled water, add 0.5 g of $MgCl_2.6H_2O$ and make up volume to one litre.
- *Ammonium hydroxide-ammonium chloride buffer (pH 10):* Dissolve 76.5 g of NH_4Cl salt in 570 ml of ammonia solution and make up volume to 1litre, adjusting pH to 10.
- *Eriochrome black T indicator:* dissolve 0.5 g of EBT dye and 4.5 g of hydroxylamine hydrochloride in 100 ml of 95% ethanol.
- *Sodium hydroxide (1N):* Dissolve 40 g of NaOH in distilled water and make up volume to one litre.
- *Murexide indicator:* Mix 200 mg ammonium purpurate with 100 g of NaCl.

Procedure (Ca^{2+} + Mg^{2+})

- Take 5 ml of water sample in a conical flask of 100 ml.
- Add 1 ml buffer and 3-4 drops of EBT indicator.
- Titrate with EDTA solution till the colour changes from wine red to sky blue.

Calculation

$$Ca^{2+} + Mg^{2+} \text{ me/l} = \frac{N \times V}{\text{ml of sample}} \times 1000$$

Where
N = Normality of EDTA solution.
V = Volume of EDTA solution used in titration.

Procedure (Ca)

- Take 5 ml of water sample in a conical flask of 100 ml.
- Add 1 ml NaOH solution and pinch of murexide indicator.
- Titrate with EDTA solution till the colour changes from pink to purple.

Calculation

$$Ca^{2+} \text{ me/l} = \frac{N \times V}{\text{ml of sample}} \times 1000$$

Where
N = Normality of EDTA solution.
V = Volume of EDTA solution used in titration.

Mg^{2+} me/l = (Ca^{2+}+Mg^{2+}) – Ca^{2+}

Mg^{2+} mg/l = Mg^{2+} me/l x 12.16

Ca^{2+} mg/l = Ca^{2+} me/l x 20.04

Hardness,

mg equivalent $CaCO_3$/l = 2.497 (Ca, mg/l) + 4.118 (Mg, mg/l)

mg equivalent $CaCO_3$/l = (Ca + Mg, me/l) x 50.05

Hardness 1 grain (gr)/gallon (gal) = Hardness mg/l / 17.1

C4e. Sodium and Potassium (Na^+ and K^+)

Sodium and potassium in water can be determined by flame photometric method or by AAS.

Reagents

- *Standard sodium solution (100 ppm):* Dissolve 0.2543 g of AR grade NaCl salt in distilled water and make up volume to one litre. Further dilute to get a series of 10, 25, 50 and 75 ppm.
- *Standard potassium solution (100 ppm):* Dissolve 0.1908 g of AR grade KCl salt in distilled water and make up volume to one litre. Further dilute to get a series of 10, 25, 50 and 75 ppm.

Procedure

- Filter a portion of water sample through Whatman # 42 filter paper.
- Calibrate flame photometer with standard solutions of Na or K.
- Aspirate the sample and record the reading.
- These concentrations should be divided by equivalent weight of Na (23) and K (39) to convert them into me/l.

Calculations for sodium hazard parameters

Residual Sodium Carbonate (RSC)

RSC me/l = $(CO_3^{2-} + HCO_3^-) – (Ca^{2+} + Mg^{2+})$

Sodium Adsorption Ratio (SAR)

$$SAR = \frac{Na}{\sqrt{\frac{Ca + Mg}{2}}}$$

References

Buurman P., B. Van Langer and E.J. Velthrost, 1996. Manual for soil and water analysis. Backhuys Publishers, Leiden, The Netherlands.

Dhyan Singh, P.K.Chhonkar, R.N. Pandey, 1999. Soil, plant & water analysis - A method manual. IARI, New Delhi.

APHA, 1998. Standard methods for the examination of waters and wastewaters. APHA-AWWA-WEF, Washington, DC.

C5. Phosphorus

Phosphorus occurs in natural waters and in wastewaters almost as phosphate. Phosphorus is essential to the growth of organisms and can be the nutrient that limits the primary productivity of a body of water. It can be determined by following methods.

C5a. Vanadomolybdophosphoric acid Colorimetric method

Ammonium molybdate reacts under acid conditions to form a heteropoly acid, molybdophosphoric acid. In the presence of vanadium, yellow vanadomolybdophosphoric acid is formed. The intensity of the yellow colour is

proportional to phosphate concentration. Concentration ranges for different wavelengths are:

P Range (mg/l)	Wavelength (nm)
1.0 – 5.0	400
2.0 – 10	420
4.0 – 18	470

Reagents

- *Phenolphthalein indicator.*
- *Acids:* HCl – H_2SO_4 (1:1), $HClO_4$, or HNO_3 may be substituted for HCl.
- *Solution A:* Dissolve 25 g of ammonium molybdate in 300 ml warm distilled water and cool it.
- *Solution B:* Dissolve 1.25 g of ammonium metavandate in 300 ml boiled distilled water. Cool and add 330 ml conc. HCl. Cool solution B and mix with solution A and make to volume one litre.
- *Standard P solution:* Dissolve 0.2195 g of dried KH_2PO_4 in distilled water, acidify with 25 ml of 7N H_2SO_4 and make up the volume to one litre to get 50 mg/l P solution.

Procedure

- Place 25 ml of sample in a 50 ml volumetric flask, add 2-3 drops of phenolphthalein indicator, if red colour appear, add 1:1 HCl till the colour disappear.
- Add 10 ml of the vanadate-molybdate reagent and dilute to 50 ml.
- Mix well and read the P concentration after 10 minutes using spectrophotometer.
- Take 0, 1, 2, 3, 4 and 5 ml of the 100 mg/l P solution in 50 ml volumetric flask and develop colour in identical manner.
- Calibrate the spectrophotometer with these known P concentration and read the concentration of sample.

Calculation

$$\text{P mg/l} = \frac{R \times 50}{25}$$

Where, R is reading of spectrophotometer P mg/l.

Reference

Proft, G. 1964. Determination of total phosphorus in water and wastewater as molybdovanadophosphoric acid. Limnologica, 2:407.

APHA, 1998. Standard methods for the examination of waters and wastewaters. APHA-AWWA-WEF, Washington, DC.

C5b. Ascorbic Acid Method

Ammonium molybdate and potassium antimony tartrate react in acid medium with ortho phosphate to form a heteropoly acid- phosphomolybdic acid- that is reduced to intensely colored molybdenum blue by ascorbic acid.

Reagents

- *Sulphuric acid (5N):* Dilute 70 ml conc. H_2SO_4 to 500 ml with distilled water.
- *Potassium antimonyl tartrate solution:* Dissolve 1.3715 g of $K(SbO)C_4H_4O_6.1/2H_2O$ in 400 ml of distilled water and dilute to 500 ml.
- *Ammonium molybdate solution:* Dissolve 20 g of $(NH_4)_6Mo_7O_{24}.4H_2O$ in distilled water and makeup to 500 ml.
- *Ascorbic acid:* Dissolve 1.76 g of ascorbic acid in distilled water and dilute to 100 ml. The solution is stable for one week at 4^0C.
- *Combined reagent:* Mix the above reagents in the following manner for 100 ml of the combined reagent: 50 ml H_2SO_4, 5 ml potassium antimonyl tartrate, 15 ml ammonium molybdate and 30 ml ascorbic acid solution. Mix after addition of each reagent. This reagent is stable for 4hrs.
- *Standard P solution:* Dissolve 0.2195 g of dried KH_2PO_4 in distilled water, acidify with 25 ml of 7N H_2SO_4 and make up the volume to one litre to get 50 mg/l P solution. Further dilute 20 times to get 2.5 mg/l P solution.

Procedure

- Place 25 ml of sample in a 50 ml volumetric flask, add 2-3 drops of phenolphthalein indicator, if red colour appear, add 5N H_2SO_4 till the colour disappear.
- Add 5 ml of the vanadate-molybdate reagent and dilute to 50 ml.
- Mix well and read the P concentration after 10 minutes but no more than 30 minutes using spectrophotometer at 880 nm.
- Take 0, 1, 2, 3, 4, 5 and 6 mL of the 2.5 mg/l P solution in 50 ml volumetric flask and develop colour in identical manner.

- Calibrate the spectrophotometer with the known P concentrations and read the concentration of test sample.

Calculation

$$\text{P mg/l} = \frac{R \times 50}{25}$$

Where, R is reading of spectrophotometer P mg/l.

Reference

Edwards, G.P, A.H. Molof and R.W. Schneeman. 1965. Determination of orthophosphate in fresh and saline waters. J. Amer.Water Works Assoc. 57:917.

APHA, 1998. Standard methods for the examination of waters and wastewaters. APHA-AWWA-WEF, Washington, DC.

C6. Nitrogen

In waters and wastewaters the forms of nitrogen of greatest interest are, in order of decreasing oxidation state, nitrate, nitrite, ammonia and organic nitrogen. All these forms of nitrogen are interchangeable and component of nitrogen cycle.

C6a. Ammoniacal and Nitrate Nitrogen

Ammonia is present naturally in surface and wastewaters. Its concentration is generally low in ground waters because it adsorbs to soil particles and clay and is not leached readily from soils. Nitrate also occurs in trace amount in surface waters but may attain high levels in some ground waters.

NH_4-N and NO_3-N are estimated using MgO and Devarda's alloy. The liberated ammonia is condensed and absorbed in boric acid, which is titrated against standard acid.

Apparatus

Nitrogen distillation unit.

Reagents

- *Magnesium oxide.*
- *Devarda's alloy.*

- *2% Boric acid:* Dissolve 20 g of H_3BO_3 powder in warm distilled water and dilute to one litre.
- *Mixed indicator:* Dissolve 70 mg of methyl red and 100 mg of bromocresol green in 100 ml of ethyl alcohol. Add 10 ml of this mixed indicator to each litre of 2% boric acid solution and adjust the pH to 4.5 with dil. HCl or dil. NaOH.
- *0.01N Sulphuric acid:* Prepare approximately 0.1N H_2SO_4 by adding 2.8 ml of conc. H_2SO_4 to about 990 ml of distilled water. Standardize it against 0.1N standard NaOH solution. Dilute 10 times this 0.1N H_2SO_4 to get a strength of 0.01N.

Procedure

- Transfer 50 ml of sample in 250 ml Kjeldhal tube.
- Add a pinch of MgO (0.2 g) and immediately distilled. Collect the 30 ml of distillate in 2% boric acid.
- Remove the Kjeldhal tube and add a pinch of Devarda's alloy and again distill.
- Collect the distillate in 10 ml of 2% boric acid solution.
- First distillate is for ammonia and second is for nitrate nitrogen.
- Titrate the distillate against 0.01N H_2SO_4 solution until pink colour starts appearing..
- Run a blank without soil for each set of samples.

Calculation

$$NH_4\text{-N or } NO_3\text{-N (mg/l)} = \frac{(S - B) \times N \times 14}{\text{Sample volume (ml)}} \times 1000$$

Where
S= Volume of acid used against sample.
B= Volume of acid used against blank.
N= Normality of acid.

C6b. Nitrite Nitrogen (NO_2-N)

Nitrite (NO_2) is determined through formation of a reddish purple azo dye produced at pH 2.0 to 2.5 by coupling diazotized sulfanilamide with N-(1-naphthul)-ethylendiamind dihydrochloride (NED dihydrochloride). The applicable range of the method for spectrophotometer measurements is 10 to 1000 µg NO_2-N/l. Photometric measurements can be made in the range 5 to 50 µg N/l if a 5-cm light path and a green color filter are used. The color system obeys Beer's law up to 180 µg N/l with a 1-cm light path at 543 nm. Higher NO_2 concentrations can be determined by diluting the sample.

Interferences

Chemical incompatibility makes it unlikely that NO_2, free chlorine, and nitrogen trichloride (NCl_3) will coexist. NCl_3 imparts a false red color when color reagent is added. The following ions interfere because of precipitation under test conditions and should be absent: Sb^{3+}, Au^{3+}, Bi^{3+}, Fe^{3+}, Pb^{2+}, Hg^{2+}, Ag^{+} , chloroplatinate ($PtCl_6^{2-}$), and metavandate (VO_3^{2-}). Cupric ion may cause low results by catalyzing decomposition of the diazonium salt. Colored ions that alter the color system also should be absent. Remove suspended solids by filtration.

Storage of sample

Never use acid preservation for samples to be analyzed for NO_2^- to NO_3^- or NH_3 for short-term preservation, freeze at -20^0C or store at 4^0C.

Instrument

Spectrophotometer.

Reagents

- *Nitrite free water:* It is not known that the distilled water is free from NO_2, use either of the following procedure to prepare nitrite free water.

1. Add a small crystal each of $KMnO_4$ and either $Ba(OH)_2$ or $Ca(OH)_2$ to distilled water and redistill. Collect the distillate that is free of permanganate; a red colour with DPD reagent indicates the presence of permanganate.

2. Add 1 ml conc. H_2SO_4 and 0.2 ml $MnSO_4$ solution (36.4 g/100 ml) to one litre distilled water, and make it pink with 1-3 ml of $KMnO_4$ solution (400 mg/l). Redistill and collect the nitrite free water.

- *Colour reagent:* Add 100 ml 85% phosphoric acid and 10 g of sulfanilamide in 800 ml of nitrite free distilled water. After dissolving sulfanilamide completely, add 1 g of N-(1-naphthyl)-ethylenediamine dihydrochloride. Mix to dissolve and make up to one litre. This solution is stable for about a month when stored in a dark bottle in a refrigerator.
- *Sodium oxalate (0.05N):* Dissolve 3.350 g of $Na_2C_2O_4$ in distilled water and make up to one litre.
- *Ferrous ammonium sulphate(0.05N):* Dissolve 19.607 g of $Fe(NH_4)_2$ $(SO_4)_2.6H_2O$ and 20 ml of conc H_2SO_4 in distilled water and dilute to one litre.
- *Stock nitrite solution:* Dissolve 1.232 g of $NaNO_2$ in distilled water and dilute to one litre. Preserve with one ml $CHCl_3$. 1.00 ml = 250 µg N.

- *Standardization of stock solution:* Pipette, in a sequence, 50 ml $KMnO_4$ (0.05N), 5 ml conc H_2SO_4 and 50 ml stock NO_2 solution into a glass stoppered flask. Shake gently and warm to 70 to 80^0C on a hot plate. Discharge permanganate colour by adding sufficient (10 ml) standard sodium oxalate (0.05 N) solution. Titrate the excess $Na_2C_2O_4$ against $KMnO_4$ (0.05 N) solution, till the faint pink end point. Carry a water blank through the entire procedure.

Calculation

Calculate NO_2-N content in stock solution by the following equation.

$$A = \frac{[(B \times C) - (D \times E)] \times 7}{F}$$

Where;
A = NO_2-N mg/ml.
B = Total ml of standard $KMnO_4$ (0.05 N) used.
C = Normality of standard $KMnO_4$ (0.05 N) used.
D = Total volume of standard sodium oxalate (0.05 N) i.e 10.
E = Normality of standard sodium oxalate (0.05N).
F = ml of $NaNO_2$ stock solution taken for titration.

Each 1.00 ml of standard $KMnO_4$ (0.05 N) consumed by the $NaNO_2$ solution corresponds to 1750 µg NO_2-N.

- *Intermediate nitrite solution:* Calculate the volume, G, of stock NO_2 solution required for the intermediate NO_2 solution from G = 12.5/A. Dilute the volume G (approx 50 ml) to 250 ml with water; 1.00 ml = 50 µg NO_2-N. Prepare daily.
- *Standard nitrite solution:* Dilute 10.00 ml intermediate NO_2 solution to 1000 ml with water; 1.00 ml = 0.500 µg NO_2-N. Prepare daily.
- *Standard potassium permanganate titrant (0.05N):* Dissolve 1.6 g of $KMnO_4$ in one litre distilled water. Stable up to 7 days when stored in a brown bottle.

Procedure

- Place 25 ml of sample in a 50 ml volumetric flask, add 2 ml of colour reagent and mix well and dilute to 50 ml.
- Read the NO_2-N concentration after 10 minutes but no more than 30 minutes using spectrophotometer at 543 nm.
- Take 0, 1, 5, 10, 15, 20 and 25 ml of the 50 µg NO_2-N solution in 50 ml volumetric flask and develop colour in identical manner.
- Calibrate the spectrophotometer with these known NO_2-N concentration and read the concentration of sample.

Calculation

$$NO_2\text{-N mg/l} = \frac{R \times 50}{25}$$

Where, R is reading of spectrophotometer P mg/l.

Reference

USEPA. 1979. Methods for chemical analysis of water and wastes. Method 353.3. U.S. Environmental Protection Agency, Washington, D.C.

APHA, 1998. Standard methods for the examination of waters and wastewaters. APHA-AWWA-WEF, Washington, DC.

C6c. Nitrate Nitrogen (NO_3-N)

Use this technique only for screening samples that have low organic matter content, i.e. uncontaminated natural waters and potable water supplies. The NO_3^- calibration curve follows Beer's law up to 11.0 ppm.

Instrument

UV-Visible Spectrophotometer.

Reagents

- *Nitrite free water:* Use redistilled or distilled deionized water to prepare all solutions and dilutions.
- *Stock nitrate solution:* Dry potassium nitrate (KNO_3) in an oven at 105^0C for 24h. Dissolve 0.7218 g in water and dilute to 1000 ml; 1.00 ml = 100 µg NO_3-N. Preserve with 2 ml chloroform. This solution is stable for at least 6 months.
- *Intermediate nitrate solution*: Dilute 100 ml stock nitrate solution to 1000 ml with water; 1.00 ml = 10 µg NO_3-N. Preserve with 2 ml chloroform. This solution is stable for at least 6 months.
- *Hydrochloric acid solution 1N.*

Procedure

- Take 50 ml filtered sample, add 1 ml 1N HCl and mix thoroughly.
- Preparation of standard curve: Prepare NO_3 calibration standard in range 0 to 7 mg/l NO_3-N by diluting to 50 ml the following volumes of intermediate nitrate solution: 0,

1, 5, 10, 15, 20, 25, 30, 35 ml (Final Con. 0, 0.2, 1, 2,3, 4, 5, 6, 7 ppm). Calibrate spectrophotometer at 220 nm and read the concentration in the test sample.

Note: Use only quartz optical cell to read concentration at 220 nm wave length.

Reference

Navone, R. 1964. Proposed method for nitrate in potable waters. J. Amer. Water Works Assoc. 56:781.

APHA, 1998. Standard methods for the examination of waters and wastewaters. APHA-AWWA-WEF, Washington, DC.

C7. Oxygen

C7a. Dissolve Oxygen (modified Winkler method)

Dissolved oxygen levels in natural and wastewaters depends on the physical, chemical and biochemical activities in the water body. The analysis for DO is a key test in water pollution and waste water treatment process control.

The sample is treated with manganous sulfate, alkaline-iodide-azide reagent and finally sulfuric acid. The first two chemicals combine with dissolved oxygen to form a compound which, when acid is added, releases free iodine (from the potassium iodide). Because the amount of iodine released is equal to the amount of oxygen present, the sample can be titrated against either sodium thiosulfate or phenyl arsine oxide (PAO) to determine the amount of dissolved oxygen present.

Reagents

- *Manganous sulfate solution:* Dissolve 480 g $MnSO_4$ $4H_2O$, or 400 g $MnSO_4$ $2H_2O$, or 364g $MnSO_4$ H_2O in distilled water, filter and dilute to 1 liter. This solution should not produce a blue color with starch indicator when added to an acidified potassium iodide (KI) solution.
- *Alkaline-iodide-sodium azide solution:* Dissolve 500 g NaOH (or 700 g KOH) and 135 g NaI (or 150 g KI) in 600 to 700 ml of distilled water. After the solution has cooled, dilute to 1 liter while stirring slowly and carefully. Dissolve 10 g sodium azide (NaN_3) in 40 ml distilled water. Add the NaN_3 solution with constant stirring, to the cooled alkaline iodide solution. Allow final mixture to cool to room temperature before using. Sodium azide will decompose over time and is no good after 3 months.
- *Sulfuric acid (H_2SO_4), concentrated.*

- *Starch indicator solution:* Use either an aqueous solution or soluble starch powder. Prepare an aqueous solution by dissolving 2 g of laboratory grade soluble starch powder and 0.2 g of salicylic acid (as a preservative) in 100 ml of hot distilled water.
- *Sodium thiosulfate standard solution (0.025 N):* Dissolve 6.205 g $Na_2S_2O_3$ $5H_2O$ in distilled water. Add 1.5 ml 6 N NaOH or 0.4 g solid NaOH and dilute to 1 liter. Standardize with bi-iodate solution.
- *Standardization of sodium thiosulfate:* Dissolve approximately 2 g KI, free from iodate, in an Erlenmeyer flask with 100 to 150 ml distilled water. Add 1 ml of 6N H_2SO_4 or a few drops of concentrated H_2SO_4, then 20.0 ml standard bi-iodate solution. Dilute to 200 ml and titrate with the sodium thiosulfate titrant solution. Add starch when a pale straw color is reached and complete the titration until the blue color first disappears. If the sodium thiosulfate is of proper strength, 20.0 ml will be used in the titration. If not, adjust the solution to the proper normality.
- *Standard potassium bi-iodate solution (0.025 N):* Dissolve 812.4 mg of potassium bi-iodate ($KH(IO_3)_2$) in distilled water and dilute to 1 liter.
- *Standard phenylarsine oxide (0.025 N):* Commercially prepared 0.0250 N PAO is available from several scientific chemical supply companies. As an alternative 0.0250 N PAO may be prepared by diluting a higher normality solution. PAO is very poisonous. Care must be taken in preparation and handling of this reagent.
- *Copper sulfate-Sulfamic acid solution:* Dissolve 32 g technical grade sulfamic acid (NH_2SO_2OH), without heat, in 475 ml distilled water. Dissolve 50 g technical grade copper sulfate ($CuSO_4.5H_2O$) in 500 ml distiller water. Mix the copper sulfate and sulfamic acid solutions together and add 25 ml glacial acetic acid (CH_3COOH).

Procedure

- Fill a 300 ml BOD bottle with sample (taking special care to avoid adding air to the liquid being collected) and close it with a stopper.
- Remove the stopper and add 1 ml of the manganous sulfate solution at the surface of the liquid.
- Add 1 ml of the alkaline-potassium iodide-sodium azide solution at the surface of the liquid.
- Replace the stopper, avoid trapping air bubbles and shake well by inverting the bottle several times. Repeat shaking after floc has settled halfway. Allow floc to settle a second time.
- Add 1 ml of concentrated sulfuric acid by allowing the acid to run down the neck of the bottle above the surface of the liquid.
- Restopper and shake well until the precipitate has dissolved.
- Titrate a volume of treated sample which corresponds to 200 ml of the original sample. This volume calculated using the formula: ml of sample to titrate = 200 x

[300/(300-2)] = 201 ml. This is a correction for the loss of some sample during the addition of reagents.

- Pour 201 ml of sample from the BOD bottle into an Erlenmeyer flask.
- If the solution is reddish-brown, titrate it against 0.0250 N sodium thiosulfate or 0.0250 N PAO until the solution is a pale yellow (straw) color. Record the amount of titrant used. Add a small quantity of starch indicator and proceed to titration.
- If the solution has no reddish-brown color, or is only slightly colored, add a small quantity (approximately 1 ml) of starch indicator. If no blue color develops, there is no dissolved oxygen.
- Titrate against 0.0250 N sodium thiosulfate or 0.0250 N PAO to the first disappearance of the blue color. Record the total number of ml of sodium thiosulfate or PAO used.

Calculations

Calculate the concentration of DO in the sample using the following formula:

$$\text{mg/l DO} = \frac{\text{ml titrant} \times \text{normality of titrant} \times 8000}{\text{Volume of sample}}$$

The electrometric meter method for determination of dissolved oxygen

There are two types of DO probes available, including a laboratory probe and a field probe. Both types operate on the same principle. An advantage of the field probe is that it eliminates the need to collect, transport and then perform analysis of a sample. For the details see the section introduction of analytical instrument.

Meter calibration

Place probe in moist air calibration chamber (such as a BOD bottle filled halfway with distilled water). Do not turn on probe stirrer. Wait for 5 minutes. Measure the air temperature, look up the calibration value, correct as necessary for altitude or salinity of samples and calibrate.

References

APHA. 1998. Standard Methods for the Examination of Water and Wastewater, APHA-AWWA-WEF, 20th Edition, Method 4500-O.

USEPA. 1979. Methods for Chemical Analysis of Water and Wastes, U.S. EPA 600/4-79-020, March 1979, Method 405.1.

C7b. Biochemical Oxygen Demand (BOD)

The Biochemical Oxygen Demand test measures the ability of naturally occurring microorganisms to digest organic matter, usually in a 5 day incubation at 20º C, by analyzing the depletion of oxygen. This measures biodegradable organic matter. This is normally expressed as O_2 mg/l.

The Biochemical Oxygen Demand test provides a quantitative measure of the amount of oxygen required to maintain the growth and activities of the biological organisms responsible for the aerobic digestion of the organic and putrescible matter in the liquid at a set temperature for a set amount of time in the dark.

The DO is measured at the beginning and recorded. After five days the DO is again determined. The BOD is then calculated on the basis of reduction of DO and the size of the sample. Measurement of DO can be made by using either an electrode and meter, or the Winkler titration.

Reagents

- *Phosphate buffer solution:* Dissolve 0.85 g KH_2PO_4 (potassium phosphate monobasic), 2.175 g K_2HPO_4 (potassium phosphate dibasic) or 2.85 g $K_2HPO_4 \cdot 3H_2O$ (potassium phosphate dibasic trihydrate), 3.34 g $Na_2HPO_4 \cdot 7H_2O$ (sodium phosphate dibasic heptahydrate) or 1.77 g Na_2HPO_4 (sodium phosphate dibasic), and 0.17 g NH_4Cl (ammonium chloride) in 100 ml reagent water. Adjust pH 7.2.
- *Magnesium sulfate solution:* Dissolve 1.10 g $MgSO_4$ or 2.25 g $MgSO_4 \cdot 7H_2O$ in reagent water, dilute to 100 ml.
- *Calcium chloride solution:* Dissolve 2.75 g $CaCl_2$ in reagent water and dilute to 100 ml.
- *Ferric chloride solution:* Dissolve 0.025 g $FeCl_3 \cdot 6H_2O$ in reagent water and dilute to 100 ml.
- *Potassium hydrogen phthalate standard:* Dissolve 300.0 mg dried potassium hydrogen phthalate in reagent water and dilute to 1000 ml.
- *Glucose-glutamic acid (G/GA) standard:* Dissolve 150.0 mg dried glucose (or dextrose) and 150.0 mg dried glutamic acid in 1000 ml reagent water.
- *Dilution water:* 1 ml each of the phosphate buffer, magnesium sulfate, calcium chloride and ferric chloride solutions for each liter of reagent water. The water is then aerated and allowed to sit for a period of 24 to 48 hours.

BOD_5 Procedure

- Two BOD bottles (300 ml) will be used for the blank, six for the three different dilutions of the sample, two for a duplicate of one of the dilutions and two each for the G/GA (glucose-glutamic acid) and KHP (potassium hydrogen phthalate).

- Add some dilution water to each BOD bottle, and then add the necessary amount of well mixed sample using a serological pipette with a wide opening tip.
- Fill each bottle to the top with dilution water. Stopper each BOD bottle so that no bubbles are visible in the sample. Place a water seal on each bottle and a plastic cap over the stopper.
- Place one set of bottles in the incubator in the dark for 5 days at 20 ± 1º C. Record the temperature, date and time of the beginning of the incubation on the bench sheet. Determine and record the DO on the duplicate set of bottles with the Winkler titration.
- At the end of the incubation period, remove the BOD bottles from the incubator and determine the DO on each bottle with the Winkler titration.
- Calculate the BOD for the samples.

Seeding

- Requirement to Seed: Samples which do not already contain enough of the proper bacteria can be analyzed for BOD_5 only after addition of "seed." Seed is nothing more than a solution containing a sufficient population of suitable bacteria.
- Sources of Seed: Effluent from a domestic treatment plant also may be the best seed for samples. Many commercial labs and some industrial discharger labs prefer to use artificial seed, such as Polybac, Polyseed, or BioSystems.

Calculation

BOD_5 - Not Seeded = DF X (DO_0 - DO_5)

Where; DF= dilution factor, DO_0 = Initial DO, DO_5 = DO after 5 days.

BOD - Seeded.

If samples are seeded, the total DO depletion over the 5-day incubation is caused by both the sample and the seed.

Seed Ratio Factor (Bottle) = f = Vol Seed sample / Vol Seed Control.

To determine the BOD_5 for seeded samples, the contribution of the seed to total DO depletion in the incubated sample must be taken into consideration. This is done by multiplying the difference between the initial DO of the seed control bottle (B_1 in Equation) and the final DO (B_2) by the seed ratio factor "f." BOD_5 for seeded samples is then calculated as

$BOD_5 = DF \times (DO_0 - DO_5) – f \times (B_1 - B_2)$.

Table BOD_5 Dilutions

Sample (ml) added to 300-mL BOD Bottle	Expected BOD_5 Minimum	Range (mg/l) Maximum	Dilution Factor
0.5	1,200	3,400	600
1	630	1,800	300
3	210	560	100
6	105	280	50
9	70	187	33.3
12	53	140	25
15	42	112	20
18	35	94	16.7
24	26	70	12.5
30	21	56	10
45	14	37	6.67
60	11	28	5
75	8	22	4
150	4	12	2
300	2	6	1

Reference

APHA, 1998. Standard methods for the examination of waters and wastewaters. APHA-AWWA-WEF, Washington, DC.

C7c. Chemical Oxygen Demand (COD) Open Reflux Method

The COD test will give a good estimate of the first stage oxygen demand for most wastewaters. An advantage of the COD test over the biochemical oxygen demand (BOD) test is 2 to 3 hours versus 5 days. The COD test also is used to measure the strength of wastes that are too toxic for the BOD test. The COD test should be considered an independent measurement and not a quick substitute for the BOD test. The COD is usually higher than the BOD, but the amount will vary from waste to waste. The COD test should be considered an independent measurement of organic matter in a sample rather than a substitute for the BOD test.

Chemical Oxygen Demand measures the ability of hot chromic acid solution to oxidize organic matter. This analyzes both biodegradable and non-biodegradable (refractory) organic matter.

In some industrial effluents (pretreatment program), BODs can be higher than CODs (for example, some effluents which are high in sugars, as can be found in the bakery industry, or soda bottling. you must first establish the COD/BOD correlation for your wastewater. The benefit of using COD is that you get your results in less than 3 hours.

Chemical oxygen demand (COD) is used as a measure of oxygen requirement of a sample that is susceptible to oxidation by strong chemical oxidant. The dichromate reflux method is preferred over procedures using other oxidants (eg. potassium permanganate) because of its superior oxidizing ability, applicability to a wide variety of samples and ease of manipulation. Oxidation of most organic compounds is 95-100% of the theoretical value.

Apparatus

- 500 ml Erlenmeyer flask with standard (24/40) tapered glass joints.
- Friedrichs reflux condensers (12-inch) with standard (24/40) tapered glass joints.
- Electric hot plate or six-unit heating shelf.
- Burette, 50 ml - 0.1 ml accuracy.
- Volumetric flasks (1000 ml capacity).
- Magnetic stirrer and stirring bars.

Reagents

- *Potassium dichromate (0.25N):* Dissolve 12.259 g of oven-dried ($K_2Cr_2O_7$) in distilled water and dilute to 1 litre volume in a volumetric flask.
- *Sulphuric acid / silver sulphate solution:* Add 5.5 g of Ag_2SO_4 / kg H_2SO_4 and mix until the silver sulphate goes into solution.
- *Mercuric sulphate (HgSO4):* Crystals or powder.
- *Ferrous ammonium sulphate (0.025N):* Dissolve 98 g of (FAS) $[Fe(NH_4)_2(SO_4)_2]$ in distilled water. Add 20 ml of conc. H_2SO_4. Cool and dilute to exactly 1 litre in a volumetric flask using distilled water.
- *Ferroin indicator :* Dissolve 1.485 g of 1,10-phenanthroline monohydrate and 0.695 g of $FeSO_4.7H_2O$ in distilled water and dilute to 100 ml. Alternatively, this indicator may be purchased as Ferroin Indicator from most scientific suppliers.

Procedure

- Place a 50ml sample in a 500ml refluxing flask and add 5 to 7 glass boiling beads.
- Add 1 g of mercuric sulphate ($HgSO_4$), 5ml of concentrated sulphuric acid / silver sulphate solution, and mix until the $HgSO_4$ is in solution.
- Add 25 ml of 0.25 N potassium dichromate ($K_2Cr_2O_7$) and mix.
- Add while mixing, an additional 70 ml of concentrated sulphuric acid-silver sulphate solution.
- After thorough mixing, attach the flask to the reflux condenser, apply heat, and reflux for 2 hours.
- A reagent blank containing 50ml of distilled water treated with the same reagent as the sample should be refluxed with each set of samples.

- After cooling remove flask from the condenser and dilute to a final volume of approximately 350 ml with distilled water.
- Add 4 to 5 drops of Ferroin indicator and a magnetic stirring bar.
- Place flask on a magnetic stirrer and rapidly titrate with 0.25 N ferrous ammonium sulphate to the first red-brown endpoint.

Calculation

$$\text{COD (mg/l)} = \frac{(A - B) \times N}{\text{ml of sample}} \times 8000$$

Where:
A= ml FAS used for blank.
B = ml FAS used for sample.
N = normality of FAS titrant .

Reference

Pitwell, L.R. 1983. Standard COD. Chem. Brit. 19:907.

APHA, 1998. Standard methods for the examination of waters and wastewaters. APHA-AWWA-WEF, Washington, DC.

C8. Effect of water quality on human health

The effect of toxic contaminants (metals, organic compounds, micro organisms) on human health can be classified as either acute or chronic. The reaction to a substance causing serious illness or death in an individual within 48 hours after exposure is considered **acute toxicity**. On the other hand **chronic toxicity** is a longer term effect on health due to frequent exposure to small amounts of a toxic substance. Chronic reactions to chemicals are difficult to study and our knowledge of the chronic toxic effects of nearly all chemicals is very poor. Examples of chronic health effects would be kidney and liver disease, cancer, mental illness, etc.

Several metal ions such as sodium, potassium, magnesium, and calcium are essential to sustain biological life. At least six additional metals, mainly transition metals, are also essential for optimal growth, development, and reproduction, i.e. manganese, iron, cobalt, copper, zinc, and molybdenum.

An element which is required in amounts smaller than 0.01% of the mass of the organism is called a **trace element**. Only manganese, iron, cobalt, copper, zinc, and molybdenum ions are in small enough quantities to be considered trace elements. Trace metals function mostly as catalysts for enzymatic activity in human bodies. However, all

essential trace metals become toxic when their concentration becomes excessive. Usually this happens when the levels exceed by 40 to 200 fold those required for correct nutritional response.

Drinking water containing the above trace metals in very small quantities may actually reduce the possibility of deficiencies of trace elements in the diet. However, in some cases, if the metal ion is present in the water supply, there is a danger of overdose and toxic effect.

In addition to the metals essential for human life, water may contain toxic metals like mercury, lead, cadmium, chromium, silver, selenium, aluminium, arsenic, and barium. These metals can cause chronic or acute poisoning and should be eliminated from the drinking water if possible.

Drinking water is regulated by guidelines which establish the maximum contamination levels. Most of these levels allow a sufficient margin of safety; however, one must remember that acceptable contaminant levels vary widely among individuals and population groups. For example, high sodium, which may be harmless for many people, can be dangerous for the elderly, hypertensives, pregnant women, and people having difficulty in excreting sodium.

Specific conductance--Also called conductivity, this is a measure of all the dissolved constituents in solution and is also referred to as the salinity, salt content, the total mineral content, or "alkali" content. Due to the many different constituents that make up the total mineral content, it is difficult to set a standard for human consumption. Conductivities range from less than 2 for distilled water to more than 2000 for highly saline waters.

Nitrate plus Nitrite--Levels greater than 10.0 mg/l pose an immediate threat to infants under six months of age because they may lead to a blood poisoning known as methemoglobinemia. Nitrate in well water may indicate contamination from agricultural runoff or septic systems.

Fluoride--A fluoride concentration between 0.7 to 1.5 mg/l is effective in the prevention of dental caries. Excessive fluoride (greater than 2.4 mg/l) may produce fluorosis (mottling of the teeth). The degree of fluorosis generally increases as fluoride concentration increases above 2.4 mg/l.

Hardness--In most water nearly all of the hardness is due to calcium and magnesium. Calcium and magnesium react with soap to form precipitates which increase soap consumption, and react with certain constituents to form scale. As a general rule, a value less than 60 is considered soft, and values above 200 are considered very hard.

Alkalinity--The alkalinity of water is a measure of its capacity to neutralize acids and is due primarily to the presence of bicarbonates. The acceptable alkalinity for municipal water supplies is generally between 30 and 500 mg/l as $CaCO_3$, but there are many water supplies above and below these limitations. Waters with alkalinity greater than 500 mg/l as $CaCO_3$ have objectionable tastes.

Chloride--chloride salts in excess of 100 mg/l give a salty taste to water. When combined with calcium and magnesium, chloride may increase the corrosive activity of water. It is recommended that the chloride content should not exceed 250 mg/l.

Sulfate--sulfate in water containing calcium forms hard scale in steam boilers. In large amounts, sulfate in combination with other constituents gives a bitter taste to water. Concentrations above 250 mg/l may have a laxative effect, but 500 mg/l is considered safe.

Aluminium--High aluminium levels are associated with premature senile dementia (Alzheimer's disease) and two other types of dementia as well.

Arsenic--Minor symptoms of chronic arsenic poisoning are similar to those of many common ailments, making actual arsenic poisoning difficult to diagnose. This type of poisoning can make people tired, lethargic, and depressed. Other symptoms are white lines across the toenails and fingernails, weight loss, nausea and diarrhoea alternating with constipation, and loss of hair. Arsenic is highly toxic and unfortunately widespread in the environment due to its natural occurrence and formerly extensive use in pesticides.

Barium--High levels of barium can have severe toxic effects on the heart, blood vessels, and nerves. It is capable of causing nerve blocks at high doses. 550 to 600 mg is a fatal dose for humans.

Cadmium--Acute cadmium poisoning symptoms are similar to those of food poisoning. Up to 325 mg of cadmium is not fatal but toxic symptoms occur at 10 mg. It is associated with kidney disease and linked to hypertension. There is also some evidence that cadmium can cause mutations.

Calcium--Low calcium intake can be related to hypertension and cardiovascular disorders. There is a link between low calcium intake and osteoporosis. With a low level of calcium in the diet, drinking water may provide a significant portion of the daily calcium requirement.

Chromium--It has been shown that freshwater and saltwater aquatic life can be adversely affected by the presence of chromium. The effect of chromium in drinking

water has not been thoroughly investigated. However, chromium is known to produce lung tumours when inhaled.

Copper--Copper deficiency causes anaemia, loss of hair pigment, growth inhibition, and loss of arterial elasticity. High levels of vitamin C inhibit good copper absorption. However, water containing amounts higher than 1 mg/l is likely to supply too much of this metal. One milligram per litre is also a taste threshold for the majority of people. Copper is highly toxic and very dangerous to infants and to people with certain metabolic disorders. Uptake of copper is also influenced by zinc, silver, cadmium, and sulphate in the diet.

Iron--The presence of iron in drinking water cause unpleasant taste and may increase the hazard of pathogenic organisms, since most of these organisms need iron to grow. More than about 0.3 mg/l of iron stains laundry and utensils reddish brown. The bioavailability of iron in drinking water has not been well researched. It is known that iron influences the uptake of copper and lead.

Lead--Lead can occur naturally, or result from industrial contamination, or be leached from lead pipes in some water systems. If the plumbing contains lead, higher levels will be detectable in the morning after water has been standing in pipes throughout the night. Lead is a cumulative poison. Lead poisoning is difficult to distinguish in its early stages from minor illness. Early reversible symptoms include abdominal pains, decreased appetite, constipation, fatigue, sleep disturbance, and decreased physical fitness. Long term exposure to lead may cause kidney damage, anaemia, and nerve damage including brain damage and finally death. The EPA has set an action level of 0.015 mg/l Lead in public water supplies.

Magnesium--An average adult ingests as much as 480 mg of magnesium daily. Any excess amounts are quickly expelled by the body. No upper limit has been set for this metal in drinking water. It can, however, create a problem for people with kidney disease. They may develop toxic reactions to high levels of magnesium, including muscle weakness, coma, hypertension, and confusion.

Manganese--Excess manganese in a diet prevents the use of iron in the regeneration of blood hemoglobin. Large doses of manganese cause apathy, irritability, headaches, insomnia, and weakness of the legs. Psychological symptoms may also develop including impulsive acts, absent-mindedness, hallucinations, aggressiveness, and unaccountable laughter. Finally, a condition similar to Parkinson's disease may develop.

Mercury--Mercury poisoning symptoms include weakness, loss of appetite, insomnia, indigestion, diarrhea, inflammation of the gums, black lines on the gums, loosening of teeth, irritability, loss of memory, and tremors of fingers, eyelids, lips, and tongue. At

higher levels, mercury produces hallucinations, manic-depressive psychosis, gingivitis, sialorrhea, increased irritability, muscular tremors, and irreversible brain damage.

Selenium--One recognised effect of selenium poisoning is growth inhibition. There is some evidence that selenium is related to skin discoloration, bad teeth, and some psychological and gastrointestinal problems. On the other hand, a small amount of selenium has been found to be protective against other heavy metals like mercury, cadmium, silver, and thallium.

Silver--The first evidence of excess silver intake is a permanent blue-gray discoloration of the skin, mucous membranes, and eyes. Large doses of silver can be fatal.

Sodium--The fact that some patients with heart disease have difficulty in excreting sodium and are put on a low sodium diet has led to the idea that sodium is bad for the heart. However, studies show no correlation between sodium concentration and cardiovascular disease mortality. On the contrary, beneficial correlations for sodium have been reported. Areas where water is hard, highly mineralized, and also high in sodium tend to have lower cardiovascular death rates. This does not contradict the fact that in some individuals the lowering of sodium in a diet is effective in lowering the blood pressure. Depending on age, general health, and sex, sodium may present a problem in drinking water. If the sodium in water exceeds 20 mg/l, it is advisable to contact the family physician for an opinion.

Organic compounds--Many industrial organic substances found in water (alomethanes, polychlorinated biphenyls or PCBs, polychlorinated phenols or PCPs, dioxin, polyaromatic hydrocarbons or PAHs) can cause death or reproductive failure in fish, shellfish, and wildlife. In addition, they can accumulate in animal and fish tissue, be absorbed in sediments, or find their way into drinking water supplies, posing long-term health risks to humans. For most of them high concentration symptoms include nausea, dizziness, tremors, and blindness.

Bacterial contamination--The main indicator of the sanitary quality of drinking water is the coliform bacteria count (MCL = average of 1 per 100 ml). A high count of these bacteria is an indication of contamination from a septic system or other fecal pollution source. The presence of coliform bacteria, which can be found in the feces of humans and animals, indicates that there is a high probability of other pathogenic organisms (disease causing germs) present. When water is contaminated with a surface drainage, noncoliform bacteria may also be present in large numbers. This type of contamination may not be harmful since there is only a small probability that drainage water contains pathogenic organisms. However, if the count of noncoliform bacteria is more than 200 per 100 ml, water is also considered to be poor quality.

Chlorination followed by dechlorination is the most common solution for potential bacteria in the water supply. Mineral and chemical problems found in an individual home water supply are usually a more common concern than bacteria and often require other treatment.

Source

http://www.bae.ncsu.edu/bae/programs/extension/wqg/smp-18/drinkwater/drinkwater.html.

www.italocorotondo.it/tequila/module2/pollution/pollutants_human_health.htm.

U.S. Environmental Protection Agency; http://www.epa.gov/safewater/mcl.html.

D. PLANT ANALYSIS

D1. Sampling and Processing

Years of research in analytical chemistry have produced reliable sampling criteria and procedures for most of the world's commercial crops/plants. Leaves are most commonly chosen, but *petioles* are selected in certain cases, e.g., cotton, sugarbeet. *Seeds* are rarely used for analysis, except for assessing of B toxicity and Zn and P deficiency in certain grain crops. In some cases, e.g., cereals, the entire above-ground young plants are sampled.

When more than one plant is sampled, include enough plants in sample to ensure that it adequately represents average composition of entire lot of plants sampled. Sampling depends upon purpose and type of analyses for which sample is taken. Damaged or diseased leaves are excluded, and plants should not be sampled when it is under moisture or temperature stress.

Plant samples should be transported to the laboratory immediately in properly labelled paper bags that allow for transpiration, this reduces the possibility of rotting. Following are the steps for processing the plant sample:

1. Cleaning plant tissue to remove dust, pesticide and fertilizer residues normally by washing the plants with de-ionized water or with 0.1 - 0.3 % P-free detergent, followed by de-ionized (DI) water. If not essentially required, samples for soluble element determination may not be washed, particularly for long periods. However, samples for total iron analysis must be washed.
2. Immediate drying in an oven to stop enzymatic activity, usually at 65°C for 24 hours.
3. Mechanical grinding to produce a material suitable for analysis, usually to pass a 60-mesh sieve; stainless steel mills are preferable, particularly when micronutrient analysis is involved.
4. Final drying at 65°C of ground tissue to obtain a constant weight upon which to base the analysis.

D2. Sample preparation for P, K, macro and trace elements

For the release of mineral elements from plant tissues, dry ashing and wet oxidation are the two widely adopted methods. Dry ashing is carried out usually at an ignition temperature of 550^0 to 600^0C followed by its extraction in dil. HCl or H_2SO_4 for determining various elements. Ashing leads to considerable volatilization loss of P, K, S and Cl. For this reason and being comparatively more time-taking, dry ashing is only occasionally adopted. Wet oxidation employs oxidizing acids like HNO_3-H_2SO_4-$HClO_4$ tri-acid mixture or HNO_3-$HClO_4$ di-acid. Use of $HClO_4$ avoids the volatilization loss of P,

K and S and provides a clear solution while H_2SO_4 helps completing oxidation. The wet oxidation method being easier, less time-consuming and convenient is given below.

Tri-acid digestion

Tri-acid mixture: Mix AR grade conc. HNO_3, H_2SO_4 and $HClO_4$ in 10:1:4 ratio and cool.

Procedure

- Transfer 0.5 or 1.0 g of dried and processed plant sample to a 250 ml conical flask.
- Add 5 ml of conc. H_2SO_4.
- Keep a glass funnel on the flask, place it on a water bath and heat at 100^0C for about 30 minute.
- Cool and add 5 ml of tri-acid mixture.
- Heat at 180-200^0C on hot plate until the dense white fumes are evolved and transparent white contents are left.
- Cool and add about 50 ml of double distilled water and filter into 100 ml volumetric flask, giving 3-4 washing. Finally make the volume 100 ml.
- Use filtrate for analysis.

Di-acid digestion

Di-acid mixture: Mix Ar grade conc. HNO_3 and $HClO_4$ in 9:4 ratio and cool.

Procedure

- Transfer 0.5 or 1.0 g of dried and processed plant sample to a 150 ml conical flask.
- Add 5 mlof conc. HNO_3, place a funnel on the flask and leave for about 6-8hr.
- After pre-digestion, add 10 ml of di-acid mixture.
- Heat at 180-200^0C on hot plate until the dense white fumes are evolved and transparent white contents are left.
- Cool and add about 25 ml of double distilled water and filter into 100 ml volumetric flask, giving 3-4 washing. Finally make the volume 100 ml.
- Use filtrate for analysis.

Microwave digestion

- Weigh 0.30 to 0.5 g sample of plant in MF vessels of microwave and add 3 ml conc. HNO_3, 3 ml H_2O_2 and 0.5 ml of HCL. Insert the vessels into the rotor of microwave.
- Set the microwave system as; Temp.100^o C/ vessel, Ramp-10 minute, Hold- 30 minute and cooling 30 minute.

- After cooling makeup volume to 50 ml.
- Determine P, K, Na, Ca, Mg, Al, B, Fe, Mn, Cu, Zn, Cd, Ca, Ni, Pb, Co, Mo, Si, S As, Ti, Be, Sr, Rb, Li, V, and Ba in the supernatant by ICP-AES or AAS.

Reference

Dhyan Singh, P.K. Chhonkar and R.N. Pandey. 1999. Soil plant water analysis - A method manual. IARI, New Delhi.

Miller, Robert O. 1998. Microwave digestion of plant tissue in a closed vessel. In Y.P. Kalra (ed.) Handbook and Reference Methods for Plant Analysis. CRC Press, New York.

AOAC. 1990. Official Methods of Analysis. Association of Analytical Chemists, Virginia, USA. Method 922, 975.

D3. Trace elements

Na, K, Zn, Fe, Cu, Al, Be, P, Si, Ti, V, Ni and Mn in acid digest of plant samples can be determined by flame photometric method or AAS.

D4. Calcium and Magnesium

These elements can be determined in the di-acid digest of plant sample either using AAS or versenate titration. When using AAS, lanthanum must be added in the sample. Addition of 0.1-1.0% lanthanum prevent the interference of Al, Be, P, Si, Ti, V and Zn.

D5. Phosphorus

Phosphorus in plant tissue can be determined in the acid digest by Vanadomolybdophosphoric yellow colour method.

Vanadomolybdophosphoric acid Colorimetric method

Ammonium molybdate reacts under acid conditions to form a heteropoly acid, molybdophosphoric acid. In the presence of vanadium, yellow

vanadomolybdophosphoric acid is formed. The intensity of the yellow colour is proportional to phosphate concentration. Concentration ranges for different wavelengths are:

P Range (mg/l)	Wavelength (nm)
1.0 – 5.0	400
2.0 – 10	420
4.0 – 18	470

Reagents

- *Solution A:* Dissolve 25 g of ammonium molybdate in 300 ml warm distilled water and cool it.
- *Solution B:* Dissolve 1.25 g of ammonium metavandate in 300 ml boiled distilled water. Cool and add 250 ml conc. HNO_3. Cool solution B and mix with solution A and make to volume one litre.
- *Standard P solution:* Dissolve 0.2195 g of dried KH_2PO_4 in distilled water, acidify with 25 ml of 7N H_2SO_4 and make the volume one litre to get 50 mg/l P solution.

Procedure

- Place 10 ml of acid digest of plant sample in a 50 ml volumetric flask, add 10 ml of the vanadate-molybdate reagent and dilute to 50 ml.
- Mix well and read the P concentration after 10 minutes using spectrophotometer at 420 nm.
- Take 0, 1, 2, 3, 4 and 5 ml of the 100 mg/l P solution in 50 ml volumetric flask and develop colour in identical manner.
- Calibrate the spectrophotometer with these known P concentration and read the concentration of sample.

Calculation

$$\text{P } \mu g/g = \frac{R \times 50}{10} \times \frac{100}{\text{Sample wt.(g)}}$$

Where,

R = reading of spectrophotometer P mg/L.
10 = volume of acid digest used for colour development.
50 = Volume make up for colour development.
100 = Volume make up after acid digestion.
g = Sample wt. (g) for acid digestion.

Reference

Piper C.S. 1966. Soil and plant analysis. Hans Publisher, Bombay.

Tandon H.L.S. 1993. Methods of analysis of soils, plants, waters and fertilizers. F.D.C.O, New Delhi.

AOAC. 1990. Official Methods of Analysis. Association of Analytical Chemists, Virginia, USA.

D6. Nitrogen and Crude Protein

Plant samples for nitrogen determination are digested in sulphuric acid at a temperature 360-410^{0}C. The rate of digestion is accelerated by using mixture of copper sulphate and sodium sulphate/potassium sulphate in the ratio of 1:9 respectively. On completion of digestion, the samples are cooled and distilled in the presence of NaOH. The distilled ammonia is collected in boric acid and titrated against standard acid.

Apparatus

Digestion block, Nitrogen distillation unit.

Reagents

- *Conc. H_2SO_4.*
- *Digestion catalyst:* Mixture of K_2SO_4/ Na_2SO_4 and Cu (9:1 respectively).
- *40 % Sodium hydroxide:* Dissolve 400 g of NaOH in distilled water and make volume one litre.
- *2% Boric acid:* Dissolve 20 g of H_3BO_3 power in warm distilled water and dilute to one litre.
- *Mixed indicator:* Dissolve 70 mg of methyl red and 100 mg of bromocresol green in 100 ml of ethyl alcohol. Add 10 ml of this mixed indicator to each litre of 2% boric acid solution and adjust the pH 4.5 with dil. HCl or dil. NaOH.
- *0.01N Sulphuric acid:* Prepare approximately 0.1N H_2SO_4 by adding 2.8 ml of conc. H_2SO_4 to about 990 ml of distilled water. Standardize it against 0.1N standard NaOH solution. Dilute 10 times this 0.1N H_2SO_4 to get strength of 0.01N.

Procedure

- Weigh 0.5 or 1.0 g of sample into digestion tube and moist with distilled water.
- Add 10 ml of conc. H_2SO_4 and 0.25 g of catalyst and place the tube in digestion unit.

- Turn the heating equipment to about 400 ^{0}C and continue heating till the mixture is transparent blue and allow to cool it.
- Add 40% NaOH in digest till the colour change blackeish and distill it.
- Collect the distillate (librated ammonia) into 10 ml of 2% boric acid solution.
- Titrate the distillate against 0.01N H_2SO_4 solution until pink colour starts appearing.
- Run a blank without sample for each set of samples.

Calculation

$$N\ (\%) = \frac{(S - B) \times N \times 1.407}{\text{Sample weight (g)}}$$

Where
S= Volume of acid used against sample.
B= Volume of acid used against blank.
N= Normality of acid.
Crude protein (CP) % = %N x 6.25.

References

Horneck, Donald A. and Robert O. Miller. 1998. Determination of total nitrogen in plant tissue. In Y.P. Kalra (ed.) Handbook and Reference Methods for Plant Analysis. CRC Press, New York.

P.C Gupta, V.K Khatta and A.B Mandal 1992. Analytical Techniques in animal nutrition. CCS Agriculture University, Hisar.

Foss Analytical. 2003. The determination of nitrogen according to kjeldahl using block digestion and steam distillation. FOSS Analytical AB, Höganäs, Sweden. AN 300.

D7. Chlorophyll Estimation

Reagent

Acetone (80%): Take 80 ml HPLC grade acetone and makeup volume to100 ml with distilled water.

Procedure

- Weigh 1 g of fresh plant material and crush in acetone with pastel and mortal.
- Make volume up to 10 ml with acetone and read the absorbance at the desired wavelength using acetone blank.

Calculation

Calculate chlorophyll a, chlorophyll b, anthocyanin and carotenoids (µg/l) in mixtures according to Arnon (1949) equations.

Total chlorophyll (µg/ml) = 20.2 (A_{645}) + 8.02 (A_{663}).

Chlorophyll **a** (µg/ml) = 12.7 (A_{663}) - 2.69 (A_{645}).

Chlorophyll **b** (µg/ml) = 22.9 (A_{645}) - 4.68 (A_{663}).

Where, A_{663} is the solution absorbance at 663 nm and A_{645} is the absorption at 645.

Unfortunately, the Arnon equations are not particularly accurate (Porra, 2002). Other equations have been derived by Porra (2000) that minimize the problems with the Arnon equations.

Chlorophyll **a** (µg/ml) = 12.25 ($A_{663.6}$) - 2.55 ($A_{646.6}$).

Chlorophyll **b** (µg/ml) = 20.31 ($A_{646.6}$) - 4.91 ($A_{663.6}$).

Total chl (µg/ml) = 17.76 ($A_{646.6}$) + 7.34 ($A_{663.6}$).

If the absorbance is greater than 0.8 then the solutions should be diluted with fresh 80% acetone and remeasured.

Sims and Gamon (2002) used a solution acteone/Tris buffer (80:20 volume; pH = 7.8) to extract tissue and derived following equations corrected for the presence of anthocyanin.

Anthocyanin (µmol ml^{-1}) = 0.08173 A_{537} - 0.00697 A_{647} - 0.002228 A_{663}.

Chl **a** (µmol ml^{-1}) = 0.01373 A_{663} - 0.000897 A_{537} - 0.003046 A_{647}.

Chl **b** (µmol ml^{-1}) = 0.02405 A_{647} - 0.004305 A_{537} - 0.005507 A_{663}.

Carotenoids (µmol ml^{-1}) = (A_{470} - (17.1 x (Chl **a** + Chl **b**) - 9.479 x anthocyanin))/119.26

Since the anthocyanin concentration estimated in the extraction medium (80% acetone) is not reliable, they report equations for determining anthocyanin in methanol/HCL/water (90:1:1, vol:vol:vol).

Anthocyanin absorbance (corrected) = A_{529} - (0.228 A_{650}) and then insert the corrected anthocyanin absorbance in the Beer-Lambert expression, A = ecl, assuming a

molar absorbance coefficient at 529 nm or 30,000 l mol^{-1} cm^{-1}.

References

Arnon, D. I. (1949) Copper enzymes in isolated chloroplasts: polyphenol oxidase in *Beta vulgaris*. Plant Physiol. 24: 1-15.

Porra, RJ (2002) The chequered history of the development and use of simultaneous equations for the accurate determination of chlorophylls *a* and *b*. Photosynthesis Research 73: 149 - 156.

Sims, DA and JA Gamon (2002) Relationships between leaf pigment content and spectral reflectance across a wide range of species, leaf structures and developmental stages. Remote Sensing of Environment 81: 337 - 354.

D8. Ether Extract/Crude Fat

Ether extract/crude fat includes all the portions of a feed which are soluble in ether. Ether is continuously volatilized at 55^0-60^0C, condensed and allowed to pass through the sample in a Soxhlet's extraction apparatus. Crude fat is a combination of simple fat, fatty acids, compound fat, neutral fat, sterols, waxes, vitamins (A, D, E, K), carotene, chlorophyll, etc.

Apparatus

Soxhlet's apparatus, thimble with cotton swab, hot air oven, hot plate, balance and desiccators.

Reagents

Petroleum ether.

Procedure

- Weigh 3-5 g of sample into a completely dried thimble.
- Remove water from the sample by placing it overnight at 105^0C in a oven. Cool it in desiccator and weigh.
- Place thimble in the Soxhlet's apparatus and fill the flask ¾ with ether. Start the water in condenser and heat the flask and set on 5-6 drops per second for four hours.
- Take out the thimble. Keep it at room temperature for evaporation of ether and then keep over night in the oven at 105^0C.
- Remove the thimble from oven, cool it in a desiccator and weigh.

Calculation

Wt. of sample = (Wt. of thimble + sample) – wt. of thimble

Wt. of fat = (Wt. of thimble + sample) - (Wt. of thimble + sample after extraction)

$$\text{Ether extract (\%)} = \frac{\text{Wt. of fat}}{\text{Wt. of sample}} \times 100$$

Note: keep this fate free sample for crude fiber estimation.

AOAC. 1990. Official Methods of Analysis. Association of Analytical Chemists, Virginia, USA. Method, 954.02.

D9. Crude Fiber (CF)

Crude fiber consists of cellulose, hemi cellulose, lignin and some minerals. The estimation is based on treating the moisture and fat free sample successively with dilute (1.25) acid and alkali.

Reagents

- *Sulfuric Acid Solution (1.25% or 0.255 N):* 1.25 g H_2SO_4/100 ml distilled water.
- *Sodium Hydroxide solution (1.25% or 0.313N):* 1.25 g NaOH/100 ml distilled water. NaOH needs to be free or nearly free from Na_2CO_3.
- *Acetone*: Extra pure or AR grade or use grade that is free from color and leaves no residue upon evaporation.

Apparatus

Spout less beakers (1000 ml), round bottom condenser, hot plate, sintered crucibles (G_1) vacuum pump, muffle furnace, oven or Fiber Analyzer.

Procedure

- Weigh 1.0 g (±0.05 g) of air-dried fat free sample and transfer it to one liter spout less beaker.
- If sample is not fat free than keep it in acetone for 10 minute and allow to air dry.
- Add 200 ml of 1.25% sulphuric acid and place on hot plate and allow refluxing for 30 minute.
- After reflux filter through muslin cloth/crucible by hot water till it is free from acid.

- Transfer the material into same beaker and add 200 ml of 1.25% NaOH and allow to reflux again for 30 minute.
- Filter in crucible with the help of vacuum pump and wash the residue with dil HCl (one time) followed by hot water till it is free from alkali.
- Dry residue with crucible in a hot air oven at 80-100^{0}C and weigh (W_2).
- Ash entire sample for 2 hours at 550°C, cool in desiccators and weigh (W_3). The loss of weight due to ignition is the weight of crude fiber.

Calculation

$$CF\ (\%) = \frac{(W2 - W3)}{W1} \times 100$$

Where
W_1 = Sample weight.
W_2 = Weight after extraction process.
W_3 = Weight after ash in muffle furnace.

Nitrogen Free Extract (NFE)

NFE (%) = 100 – (CP% + CF% + EE% + Total ash %).

Reference

www4.nau.edu/Hungate_Lab/Methods/ankomCF_methods.htm.

Foss Analytical. 2003. The determination of crude fibre (cf) in feed according to AOAC, ACCC and AOCS standards. FOSS Analytical AB, Höganäs, Sweden. AN 3428.

AOAC. 2000. Official Methods of Analysis. Association of Analytical Chemists, Virginia, USA. 15th edition. Method 920.39.

D10. Van Soest Method of Fiber Analysis

CF should contain total cellulose, hemi-cellulose and lignin but only a part of the hemi-cellulose and lignin comes out in CF fraction. The remaining portion shows as NFE, which is usually thought consisting mainly of highly digestible sugars and starch. Consequently, the hemi-cellulose, low in digestibility and lignin essentially indigestible appears in NFE fraction, thereby giving an incorrect index regarding nutritive value of a given feed. To overcome these limitations, Van Soest (1963 &1967) developed a new scheme of analysis of forage dry matter in terms of cell wall and cell constituents using

various detergents. According to this scheme, cell contents, which are soluble in neutral detergents, include lipids, sugars, organic acids, starch, soluble proteins, non-protein nitrogenous compounds and other water soluble matters. While the insoluble material known as neutral detergent fiber (NDF) comprises cell wall constituents which include hemi-cellulose, cellulose, lignin, lignified nitrogenous compounds, heat damaged proteins, keratins and silica. When NDF is treated with acid detergent, hemi-cellulose, some cell wall nitrogenous compounds and acid soluble ash pass into solution leaving all other cell wall constituents in the insoluble fraction which is named as acid detergent fiber (ADF) or lingo-cellulose. This lingo-cellulose when treated with 72% H_2SO_4 the cellulose is dissolved in the acid leaving behind the lignin and acid insoluble ash, from which lignin can be determined by loss of ignition. Detailed procedures for the estimation of these fractions are given below:

D10a. Neutral Detergent Fiber (NDF)

Apparatus

Spout less beakers (1000 ml), round bottom condenser, hot plate, sintered crucibles (G_1 grade), vacuum pump, muffle furnace, oven or Fiber Analyzer.

Reagents

- *Neutral Detergent Solution (NDS):* Add 30.0 g sodium lauryl sulfate; 18.61 g Ethylenediaminetetraacetic Disodium Salt (EDTA, Dihydrate); 6.81 g sodium tetraborate decahydrate; 4.56 g sodium phosphate dibasic, anhydrous; and 10.0 ml triethylene glycol, in 1000 ml distilled water. Agitate and heat to facilitate solubility. Check pH range to 6.9 to 7.1.
- *Sodium sulfite:* Na_2SO_3, anhydrous.
- *Acetone*: Extra pure or AR grade or use grade that is free from color and leaves no residue upon evaporation.
- *Decahydronaphthalene.*
- *n-octanol.*

Safety Precautions

- Acetone is highly flammable. Use fume hood when handling acetone and avoid inhaling or contact with skin. Make sure bags are completely dry and that all the acetone has evaporated before placing in oven.
- Sodium lauryl sulfate will irritate the mucous membranes. A dust mask and gloves should be worn when handling this chemical.

Procedure

- Weigh 0.5 g (± 0.05 g) of air-dried sample (W_2), into spout-less beaker.
- Add 100 ml of detergent solution (NDS), 0.5 g of sodium sulfite and 2 ml of decahydronaphthalene.
- Reflux for 60 minute after boiling.
- Filter through a pre weighed crucible, rinse the sample with hot water. Filter the liquid and repeat the washing process 3-5 times.
- Wash twice with acetone and suck dry.
- Allow acetone to evaporate and after that complete drying in oven at 105° C for at least 8 hours and weigh (W_3).
- Ash entire sample for 2 hours at 550°C, cool in desiccator and weigh (W_4).

Calculation

$$\textbf{NDF (\%)} = \frac{(W_3 - W_1)}{W_2} \times 100$$

$$\textbf{NDF}_{\textbf{OM}}\ \textbf{(DM basis) \%} = \frac{(W_4 - W_1)}{W_2} \times 100$$

Where
W_1 = Empty crucible weight.
W_2 = Sample weight.
W_3 = Weight after extraction process.
W_4 = Weight of Organic Matter (OM)- loss of weight on ignition.

D10b. Acid Detergent Fiber

Apparatus

Same as used in NDF estimation.

Reagents

- *Acid Detergent Solution (ADS):* Add 20 g cetyl trimethylammonium bromide (CTAB) to 1 liter 1N H_2SO_4.
- *Acetone*: Extra pure or AR grade or use grade that is free from color and leaves no residue upon evaporation**.**
- *Decahydronaphthalene.*
- *n-hexane or n-octanol.*

Safety Precautions

- Acetone is highly flammable. Use fume hood when handling acetone and avoid inhaling or contact with skin. Make sure bags are completely dry and that all the acetone has evaporated before placing in oven.
- Rubber gloves and face shield should be worn when handling sulfuric acid. Always add sulfuric acid to water. If acid contacts skin wash with copious amounts of water.
- CTAB will irritate the mucous membranes. A dust mask and gloves should be worn when handling this chemical.

Procedure

- Weigh empty crucible (W_1) record weight and tare balance.
- Weigh 0.5 g (± 0.05 g) of air-dried sample (W_2), ground to pass through a 1mm screen (2 mm screen when using a cyclone mill) in spout-less beaker.
- Add 100 ml of acid detergent solution (ADS) and 2 ml of decahydronaphthalene.
- Reflux for 60 minute after boiling.
- Filter through a pre weighed crucible, rinse the sample with hot water. Filter the liquid and repeat the washing process 3-5 times.
- Wash twice with acetone and suck dry.
- Wash with hexane if crucible contains some acetone.
- Suck the hexane and dry in oven at 105° C for at least 8 hours and weigh (W_3).
- Ash entire sample for 2 hours at 550°C, cool in desiccator and weigh (W_4).
- If doing ADL then not put in muffle furnace.

Calculation (ADF Percent)

$$\text{ADF (as-is basis)} = \frac{(W_3 - W_1)}{W_2} \times 100$$

$$\text{ADF (DM basis)} = \frac{(W_3 - W_1)}{W_2 \times DM} \times 100$$

$$\text{ADF}_{OM} \text{ (DM basis)} = \frac{(W_4 - W_1)}{W_2 \times DM} \times 100$$

Where
W_1 = Empty crucible weight.
W_2 = Sample weight.
W_3 = Weight after extraction process.
W_4 = Weight of Organic Matter (OM) - loss of weight on ignition.

D10c. Acid Detergent Lignin (ADL)

Estimation of ADL requires preparation of ADF first.

Apparatus

Same as required for ADF-NDF.

Reagent

Sulphuric acid (72%) w/w: Take 1200 ml distilled water in volumetric flask of 2000 ml and 800 ml of conc. H_2SO_4.

Procedure

- After estimation of ADF, fill the crucible with 72% H_2SO_4, stir with glass rod to smooth the paste and break the lumps.
- Refill with sulphuric acid and stir at hourly intervals as acid drains away. Give a total three addition.
- After three hours, filter off as much acid as possible. Wash content with hot water 3-5 times.
- Dry in oven at 105° C for at least 8 hours and weigh (W_5).
- Ash entire sample for 2 hours at 550°C, cool in desiccator and weigh (W_6).

Calculation (ADL Percent)

$$\textbf{ADL} = \frac{(W_5 - W_6)}{W_2} \times 100$$

Where
W_5 = Weight after lignin extraction (Crucible + Lignin).
W_2 = Sample weight at ADF initiation.
W_6 = Weight after ash (crucible + Ash).

References

AOAC. 2002. Official Methods of Analysis. 15th ed. Assoc. Offic. Anal. Chem., Arlington, VA.

Foss Analytical. 2003. The determination of acid detergent fibre (cf) in feed. FOSS Analytical AB, Höganäs, Sweden. AN 3436.

Foss Analytical. 2003. The determination of acid detergent lignin in feed. FOSS Analytical AB, Höganäs, Sweden. AN 3430.

P.C Gupta, V.K Khatta and A.B Mandal 1992. Analytical Techniques in animal nutrition. CCS Agriculture University, Hisar.

Van Soest P.J 1963, Use of detergents in the analysis of fibrous feeds. J. Assn Offic. Agr. Chem..46:829-35.

Van Soest PJ, Robertson JB and BA Lewis. 1991. Methods for dietary fiber, neutral detergent fiber and non starch polysaccharides in relation to animal nutrition. J. Dairy Sci. 74:3583-97.

Van Soest, P. J. 1967. Development of a comprehensive system of feed analyses and its application to forages. J. Anim. Sci. 26:119–128.

Van Soest, P. J. 1973. Collaborative study on acid-detergent fiber and lignin. J. AOAC Int. 56:781–784.

E. BASICS OF ANALYTICAL EQUIPMENT

E1. pH Meter

A **pH meter** is a specific type of voltmeter with a very high impedance of the input channels. The high impedance is a necessary part of the equipment because of high resistance of the pH glass electrode typically used with pH meters (usually between 20 and 100 MΩ).

First commercial pH meters were built around 1936 by Dr. Arnold Beckman in the US and by Radiometer in Denmark. Dr. Beckman's invention helped him launch the successful Beckman Instruments Company.

pH meter works on Nerns't equation and pH is defined as the negative logarithm of hydrogen ion concentration.

$pH = - \log (H^+)$

Fig. pH meter

The common pH meter has several inputs for indicator (ion-sensitive or redox) and reference electrode and temperature sensors such as thermoresistors or thermocouples.

Two electrodes are used in the determination of pH. One is the reference electrode, which provides standard voltage. It contains two layers, first layer consist of a strip of platinum sealed in glass and dipped into paste of calomel (Hg_2Cl_2, 0.1 M) and the second layer filled with saturated KCl solution. Second electrode is a glass electrode that consists of high resistance glass tube with a thin, low resistance glass bulb at the bottom. It encloses silver coated silver wire with wax insulation.

When the electrodes are dipped in the solution, the saturated solution of KCl comes out of the reference electrode through the small holes and forms an invisible ionic bridge between electrodes through which current passes. The H ions absorbed by glass electrode and electric potential develops between electrodes. This potential difference is measured in terms of pH by suitable galvanometer.

The pH scale of the device should be calibrated by at least two buffer solutions. Usually one of the buffers used for calibration has pH 7.00 and the second is selected depending on the range where the measurements are to be taken - 9.20 for basic solutions and 4.01 for acidic solutions. This correlates the measured potential of the indicator electrode with the pH scale.

General tips:

1. The electrode should always remain immersed in the water.
2. Allow the instrument to stabilize for a period of at least 30 minute before use.
3. Change the water in the beaker daily.
4. If there is strong decline or fluctuation in the reading, fill the electrode with saturated KCl solution.

E2. Conductivity Meter

A conductivity meter measures the ionic conductivity (or conversely, the resistance) of a liquid. The number it gives can not directly be related to hardness, but rather, the total ion content of the liquid. What the device usually consists of is a probe which usually has two platinum electrode plates parallel to each other and separated by some small distance. The meter is in its simplest form, a Wheatstone Bridge apparatus with a small oscillator and a readout. The oscillator generates small amplitude (about 5 to 10 mV peak-to-peak) sin wave. The meter is zeroed by dialing in a resistance on one side of the Wheatstone bridge. The resistance dialed in is then the resistance between the two plates in the probe. Relating this value to a calibration curve for the probe will give you the concentration of ionic species in solution.

Reporting the levels

Conductivity is customarily reported in micro-mhos per centimeter (µmho/cm). In the international system of units the reciprocal of the ohm is the simens (S) and the conductivity is reported as milli-siemens or desi-siemens per meter (mS/m or dS/m).

Conductivity (µS/cm) X 0.5 = TDS mg/l as NaCl

Conductivity standard solutions

Conductivity is measured against standard solution of KCl and following concentrations are used for the calibration:-

Solution type	**mass of KCl /Litre**	**Conductivity at 25°C**	**Temp. Coefficient at 25°C**
1.0 M	71.1352 g	111.3 mS/cm	1.89 %
0.1 M	7.4191 g	12.85 mS/cm	1.90 %
0.01 M	0.7452 g	1408 µS/cm	1.94 %
0.001 M	0.0753 g	146.1 µS/cm	2.04 %

The above table also can be used to calculate the conductivity at any other ambient temperature.

Example: Calculation of conductivity of 0.01 M KCl at 30°C

C 30°C = C 25°C + C 25°C X 1.94/100 X (30°C - 25°C)

= 1408 µS + 1408 X 1.94/100 X 5

= 1544.5 µS/cm

E3. UV-Visible Spectrophotometer

The instrument used in Ultraviolet-visible spectroscopy is called a ultraviolet-visible **spectrophotometer**. To obtain absorption information, a sample is placed in the spectrophotometer and ultraviolet or visible light at a certain wavelength, or range of wavelengths, is transmitted through the sample. The spectrophotometer measures how much of the light is absorbed by the sample.

The functioning of this instrument is relatively straightforward. A beam of light from a visible and/or UV light source (colored red) is separated into its component wavelengths by a prism or diffraction grating. Each monochromatic (single wavelength) beam in turn is split into two equal intensity beams by a half-mirrored device. One beam, the sample beam (colored magenta), passes through a small transparent container (cuvette) containing a solution of the compound being studied in a transparent solvent. The other beam, the reference (colored blue), passes through an identical cuvette containing only the solvent. The intensities of these light beams are then measured by electronic detectors and compared. The intensity of the reference beam, which should have suffered little or no light absorption, is defined as $\mathbf{I_0}$. The intensity of the sample beam is defined as **I**. Over a short period of time, the spectrometer automatically scans all the component wavelengths in the manner described. The ultraviolet (UV) region scanned is normally from 200 to 400 nm, and the visible portion is from 400 to 800 nm. A diagram of the components of a typical spectrometer is shown in the following diagram.

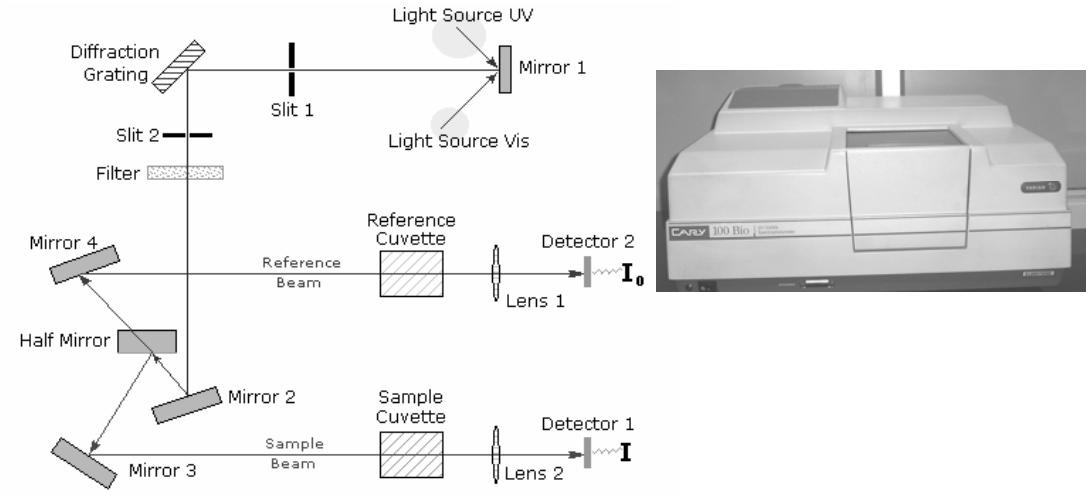


Fig. UV-Visible spectrophotometer and its flow diagram.

If the sample compound does not absorb light of of a given wavelength, $\mathbf{I = I_0}$. However, if the sample compound absorbs light then **I** is less than $\mathbf{I_0}$, and this difference may be plotted on a graph versus wavelength, as shown in the above figure.

Absorption may be presented as **transmittance** ($\mathbf{T = I/I_0}$) or **absorbance** ($\boldsymbol{A}$= log $\mathbf{I_0/I}$). If no absorption has occurred, T = 1.0 and *A*= 0. Most spectrometers display absorbance on the vertical axis, and the commonly observed range is from 0 (100% transmittance) to 2 (1% transmittance). The wavelength of maximum absorbance is a characteristic value, designated as $\boldsymbol{\lambda_{max}}$. Different compounds may have very different absorption maxima and absorbances. Intensely absorbing compounds must be examined in dilute solution, so that significant light energy is received by the detector, and this requires the use of completely transparent (non-absorbing) solvents. The most commonly used solvents are water, ethanol, hexane and cyclohexane. Solvents having double or triple bonds, or heavy atoms (e.g. S, Br & I) are generally avoided. Because the absorbance of a sample will be proportional to its molar concentration in the sample cuvette, a corrected absorption value known as the **molar absorptivity** is used when comparing the spectra of different compounds. This is defined as by Beer-Lambert law: The Beer-Lambert law states that within small ranges, the concentration of the desired compound varies linearly with the absorbance. Thus UV/VIS spectroscopy can determine the concentration of an unknown solution, based on reference molar extinction coefficients or more accurately, using a calibration curve

Molar Absorptivity, $\boldsymbol{\varepsilon} = \dfrac{A}{C \times I}$

(where *A*= absorbance, **c** = sample concentration in moles/liter & **l** = length of light path through the cuvette in cm.).

UV/Vis spectroscopy is routinely used in the quantitative determination of solutions of transition metals and highly conjugated organic compounds. It is possible to do so because transition metals are often colored because of the possibility of d-d electronic transitions within the metal atoms. Organic molecules, especially those with a high degree of conjugation also absorb light in the UV or visible regions of the electromagnetic spectrum.

E4. Flame Photometer

Flame photometers use atomic emission for the routine detection of metal salts, principally sodium (Na), potassium (K), lithium (Li) and calcium (Ca). A flame photometer is an instrument used for measuring the spectral intensity of metals present in the metallic salt.

Flame photometry, now more properly called *flame atomic emission spectrometry*, is a relatively old instrumental analysis method. Its origins date back to Bunsen's flame color tests for the *qualitative* identification of select metallic elements. As an analytical method, atomic emission is a fast, simple, and sensitive method for the determination of trace metal ions in solution. Because of the very *narrow* (*ca*. 0.01 nm) *and characteristic* emission lines from the gas-phase atoms in the flame plasma, the method is relatively free of interferences from other elements.

Theory

1. Sample solution **sprayed or aspirated** as fine mist into flame. Conversion of sample solution into an aerosol by atomizer.
2. Heat of the flame **vaporizes** sample constituents.
3. By heat of the flame + action of the reducing gas (fuel), molecules & ions of the sample species are **decomposed and reduced to give ATOMS**. *eg* $Na^+ + e^-$ --> Na
4. Heat of the flame causes **excitation** of some atoms into higher electronic states.
5. Excited atoms **revert to ground state** by **emission** of light energy, $h\nu$, of characteristic wavelength; measured by detector.

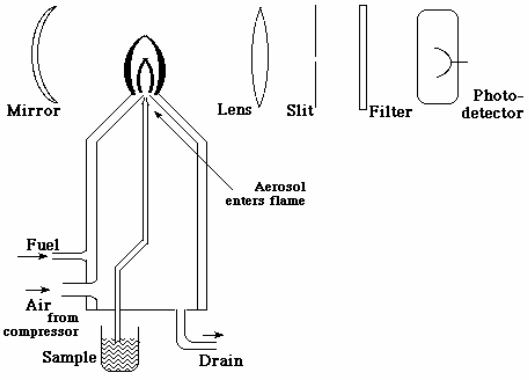
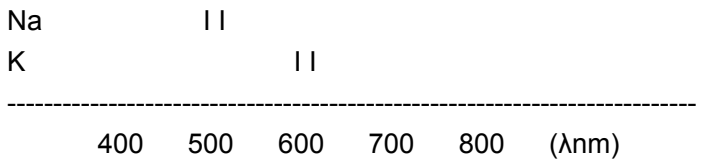


Fig. Flame photometer and its flow diagram.

Atoms in the vapour state give **LINE SPECTRA** (Not band spectra, because no covalent bonds hence no vibrational sub-levels to cause broadening).

Coloured glass filter usually able to isolate the line of analyte element if well separated from other emission lines. *eg* To measure sodium and potassium separately in samples containing both



General tips for flame photometry

1. Propane-air or natural gas-air gives good flame - strong heat, minimal background light emission. But always need to run a **solvent blank** for setting zero emission.
2. **Solutions diluted** to fall within **linear part of emission curve**. Can calibrate with standards accordingly (eg from 0.05 - 0.25 mM Na^+).
3. Use of **very low conc** Na^+ and K^+ solutions ---> problems of **avoiding contamination.** Especially Na^+, leaches slowly from glass, contact with skin.
4. **Anion and cation interference** effects can cause errors (**enhancement** or **suppression**). **"Radiation buffer"** for dilution of standards and samples to swamp out inconsistencies.
5. **Internal standard (lithium)** useful to counter random flame instability and random dilution errors.

E5. Atomic Absorption Spectrophotometer

Atomic absorption spectrophotometer (AAS) is an analytical equipment based on atomic absorption spectro-photometry and is used to measure metals in the sample. When a sample is aspirated into the instrument, it is subjected to a heavy thermal environment and as a result, 'the ground state' atoms absorb light energy of a specific wavelength and enter in to the excited sate. As the number of atoms in the light path increases, the amount of light absorbed increases in a predictable way. By measuring the amount of light absorbed, a quantitative determination of the amount of analyte element present can be made.

Every absorption spectrometer have five basic components which are (1) a light Source (cathode lamp); (2) a sample cell (absorption cell); (3) Monochromometer; (4) Detector; and (5) out put unit.

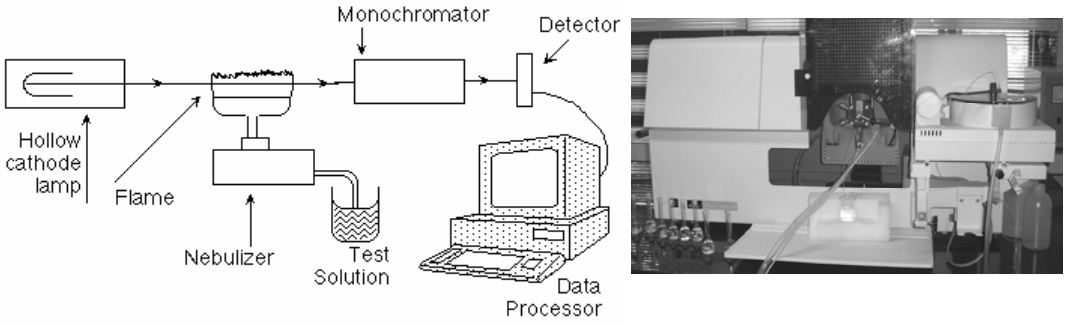


Fig. Atomic absorption spectrophotometer and its flow diagram.

Absorption in the flame is by **vapour phase atoms**, giving absorption line spectra. A continuous spectrum light source, even with high quality monochromator cannot achieve sufficiently narrow band pass width for absorption line spectra. Use of special lamps, each **emitting line spectrum** matched to the line spectrum of the analyte atoms in the flame. The type of lamp is a **hollow cathode lamp**. Different lamp for each analyte element is required, but some multi-element lamps also available.

Make up three standards.

The first one should be at the top of the linear range. The concentration of the second standard should be approximately 3 times the concentration of the first. The concentration of the third standard should be approximately 6 times the concentration of the first standard.

Characteristic Concentration vs. Detection Limit

Characteristic concentration in atomic absorption (sometimes called "sensitivity") is defined as the concentration of an element (expressed in mg/l) required to produce a signal of 1% absorption (0.0044 absorbance units). As long as measurements are made in the linear working range, characteristic concentration can be determined by reading the absorbance produced by a known concentration of the element, and solving the following equation:

Characteristic Concentration = Conc. of Std. (mg/l) X 0. 0044

The characteristic concentration check value is the concentration of element (in mg/l) that will produce a signal of approximately 0.2 absorbance units under optimum conditions at the wavelength listed.

E6. Microwave Digestion System

Microwave digestion system is an advanced and highly sophisticated system for sample preparation, which utilize the electromagnetic radiation to achieve higher temperature for the reaction and it provides high performance, reliable quality and unrivaled safety, which is required in sample preparation in order to achieve superior analytical results. Its closed vessel technique helps to speed up reactions by allowing higher temperatures, while preventing the loss of volatile analytes. The resulting low reagent consumption saves time and money and also helps to minimize exposure to corrosive gases and hazardous solvent vapors. The magnetron of the system generates microwaves of 0.3 mm – 1.0 m wavelength having frequency of 100GHz to 300 MHz, which has strong penetrating power on the matrix to extract the analyte of interest.

This instrument is now widely used in domestic, commercial and scientific segments for a variety of purposes. In the field of research, it is specifically employed in digesting of various abiotic and biotic matrices to extract organic and inorganic chemicals.

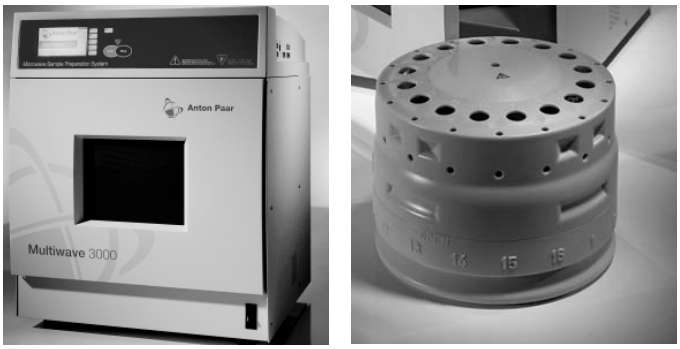


Fig. Microwave digestion system and its rotor.

E7. Auto Kjeldhal Nitrogen Analyzer

Nitrogen determination has a long history in the area of analytical chemistry. Johan Kjeldahl first introduced the Kjeldahl nitrogen method in 1883. While studying proteins during malt production, he developed a method of determining nitrogen content that was faster and more accurate than any method available at the time. Since 1883, the Kjeldahl method has gained wide acceptance and is now used for a variety of applications. Kjeldahl nitrogen determinations are performed on food and beverages, meat, feed, grain, waste water, soil and many other samples. The method has been refined and tested for a wide variety of substances and approved by various scientific associations including: AOAC, AACC, EPA, ISO, USDA.

The auto kjeldhal nitrogen analyzer consists of two separate units, block digester and distillation, titration assembly. In block digester digestion is performed at 420^0C further digested samples transferred into distillation titration assembly where librated ammonia collected in boric acid solution and titrated against standard acid. After the end of process result can be read out on display.

Fig. Automatic nitrogen analyzer with block digester.

The Kjeldahl method may be broken down into three main steps:

Digestion - the decomposition of nitrogen in organic samples utilizing a concentrated sulfuric acid. The end result is an ammonium sulfate solution.

Organic N + $H_2SO_4 \rightarrow (NH_4)_2SO_4 + H_2O + CO_2$ + other sample matrix by-products.

Distillation - Adding excess base to the acid digestion mixture to convert $(NH_4)_2SO_4$ to NH_3, followed by boiling and condensation of the NH_3 gas in a receiving solution.

$(NH_4)_2SO_4 + 2NaOH \rightarrow 2NH_3 + Na_2SO_4 + 2H_2O$

$NH_3 + H_3BO_3 \rightarrow NH_4^+: H_2BO_3^- + H_3BO_3$

Titration - To quantify the amount of ammonia in the receiving solution, the amount of nitrogen in a sample can be calculated from the quantified amount of ammonia ions in the receiving solution.

$NH_4^+: H_2BO_3^- + HCl \rightarrow NH_4Cl + H_3BO_3$

$$N\ (\%) = \frac{(S - B) \times N \times 1.407}{\text{Sample weight (g)}}$$

Where

S= Volume of acid used against sample.

B= Volume of acid used against blank.

N= Normality of acid.

If it is desired to determine % protein instead of % nitrogen, the calculated % N is multiplied by a factor, the magnitude of the factor depending on the sample matrix.

Many protein factors have been developed by AACC and AOAC for use with various types of samples such as, 6.38 for milk and dairy, 5.95 for rice, 5.70 for wheat flour and 6.25 for other grains.

E8. Fiber Analyzer

This instrument used for analysis of fiber as Crude Fiber, Neutral Detergent Fiber (NDF), Acid Detergent Fiber (ADF) & Acid Detergent Lignin (ADL).

Fiber analyzer is specifically designed for fiber determination in accordance with the Weende, van Soest and other recognized methods. There is single or sequential extractions including boiling, use of internally preheated reagents, rinsing and filtration are performed under reproducible and controlled conditions. This system is made of two units, hot extractor and cold extractor. Hot Extractor, for hot hydrolysis and extraction, featuring built-in systems for heating, filtration, automatic preheating and addition of reagents while Cold Extraction Unit, for de-fatting samples, extraction at ambient temperatures (e.g. in lignin determination) and solvent dehydration of fiber residues. Samples are handled in specially designed filter crucibles. Crucibles are used both as an integral part of the assembly during extraction, rinsing and filtration and as sample vessels during weighing, drying and ashing.

Fig. Automatic fiber analyzer.

E9. Direct Current Plasma (DCP) Emission

DCP is an atomic emission technique. Sample is aspirated into a premix spray chamber through a nebulizer using a system very similar to that for atomic absorption. However, instead of combustible gases, argon is used as a transport gas for the sample. The sample aerosol in a stream of argon is directed at a set of electrodes, across which a high voltage electrical potential is applied. The resulting electrical discharge between the electrodes supplies enough energy to ionize the argon into a ‘‘plasma’’ of positively charged argon ions and free electrons. The thermal energy of the plasma, in turn, atomizes sample constituents and creates excited state atoms, which emit their characteristic atomic emission spectra. DCP was the first plasma technique applied to routine atomic emission analyses. In its early days, it was an especially valuable complement to atomic absorption in that DCP provided good detection limits for the refractory elements, for which atomic absorption was not particularly sensitive. DCP is also capable of simultaneous multielement analysis and qualitative, as well as quantitative, analysis. DCP carries with it some significant disadvantages, however. The electrodes which form the DC arc are continually eroded and burned away during operation. This imposes a maintenance problem of continual adjustment and replacement of the electrodes. In addition, the very high temperatures for which plasmas are known are not fully realized in the DCP design. Due to a highly resistant ‘‘skin effect’’, the sample does not penetrate into the hottest part of the plasma, but is instead deflected around it. The analytical measurement normally is made just underneath the hottest part of the plasma, where temperatures are hot enough to provide good sensitivity for refractory elements but not hot enough to eliminate chemical and ionization effects. Procedures for reagent addition usually are prescribed to deal with these interferences. Because these limitations are not normally encountered with inductively coupled plasma (ICP) systems, ICP emission is normally a preferred emission technique.

E10. Inductively Coupled Plasma (ICP) Emission

Similar to DCP, ICP is an atomic emission technique using an argon plasma as an excitation source. However, the design of the source is completely different. Sample is again introduced into a premix spray chamber, where it is directed up the central tube of the ICP ‘‘torch’’. The torch consists of concentric tubes with independent argon streams flowing through each. The top of the torch is centered within a radio frequency (RF) induction coil, which is the source of energy for the system. After ignition, the plasma is propagated through inductive coupling with the RF field generated from the coil. Unlike DCP, there are no electrodes to maintain and replace. Further the ICP torch is designed specifically to promote penetration of the plasma skin by the sample, allowing sample atoms to experience the full energy of the plasma source. The high temperatures provided by the ICP provide excellent sensitivities for refractory elements

and also essentially eliminate chemical interferences. Like all emission techniques, there are no source lamps. By monitoring several wavelengths, either all at once or in a programmed sequence, many elements can be determined in one automated analysis. ICP emission, therefore, offers significant speed advantages over atomic absorption for multielement analyses. Except for the refractory elements, which may be substantially better than even graphite furnace AA, ICP detection limits are comparable to flame atomic absorption. The high temperatures of the ICP carry one disadvantage. The plasma is so effective in generating excited state species that the rich emission spectra produced increase the probability of spectral interferences. High resolution monochromators and sophisticated software for background and interelement correction are used to deal with this potential problem. Another limitation of ICP emission is the initial cost of the instrumentation. The price for basic ICP systems starts at about the same level as the prices for top-of-the-line automated AA systems. More sophisticated instrumentation can cost two to four times the price of basic systems.

E11. Inductively Coupled Plasma - Mass Spectrometry (ICP-MS)

ICP-MS is one of a growing number of ‘‘hyphenated techniques’’, where the output of one technique becomes the input of another. For ICP-MS, the ICP is used as the ion source for a mass spectrometer. The ions are then spatially separated according to their mass and charge, and measured individually. The major attractiveness of ICP-MS is its exceptional sensitivity combined with high analysis speed. For most elements, ICP-MS offers detection limits which are comparable to or better than those of graphite furnace AA. But ICP-MS can determine many elements in the time required for the determination of one element by graphite furnace AA. ICP-MS also offers the ability for isotopic analysis. As with the other techniques, ICP-MS also has its limitations. The relative newness of ICP-MS means, while the required instrumentation is well developed, many developments in analytical methodology are yet to be made. This translates into additional effort for the analyst in adapting the technique to his or her particular analytical needs. Since ICP-MS is not a spectroscopic technique, spectral interferences do not occur. Interferences from mass overlaps due to other isotopes and polyatomic species do occur, however, and may provide erroneous results unless properly corrected. The major limitation of ICP-MS at this time, however, may be its cost. ICP-MS systems typically are two to four times as expensive as basic ICP emission systems. However, the unique abilities of ICP-MS to provide graphite furnace detection limits with the analytical speed of ICP emission and to perform isotopic analysis capabilities frequently provide the justification needed to overcome cost limitations.

E12. High-performance Liquid Chromatography (HPLC)

HPLC is a form of liquid chromatography to separate compounds that are dissolved in solution. HPLC is a popular method of analysis because it is easy to learn and use and is not limited by the volatility or stability of the sample compound.

High pressure liquid chromatography was developed in the mid-1970's and quickly improved with the development of column packing materials and the additional convenience of on-line detectors. In the late 1970's, new methods including reverse phase liquid chromatography allowed for improved separation between very similar compounds. Modern HPLC has many applications including separation, identification, purification, and quantification of various compounds.

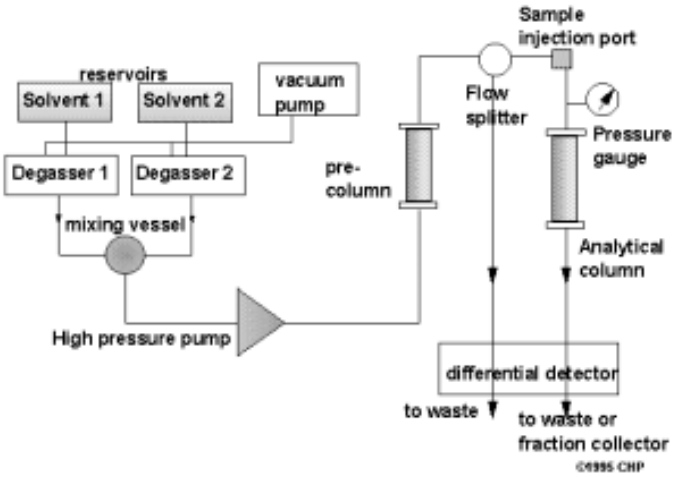


Fig. High Performance Liquid Chromatograph and its flow diagram

Although HPLC is widely considered to be a technique mainly for biotechnological, biomedical, and biochemical research as well as for the pharmaceutical industry, these fields currently comprise only about 50% of HPLC users. Currently HPLC is used by a variety of fields including cosmetics, energy, food, and environmental industries.

HPLC instrument consist of a reservoir of mobile phase, a pump, an injector, a separation column, and a detector. Compounds are separated by injecting a plug of the sample mixture onto the column. The different components in the mixture pass through the column at different rates due to differences in their partitioning behavior between the mobile liquid phase and the stationary phase.

The **mobile phase** in HPLC refers to the solvent being continuously applied to the column, or stationary phase. The mobile phase acts as a carrier for the sample solution. A sample solution is injected into the mobile phase of an assay through the

injector port. As a sample solution flows through a column with the mobile phase, the components of that solution migrate according to the non-covalent interactions of the compound with the column. There are several types of mobile phases, these include: isocratic, gradient, and polytypic.

In **isocratic elution** compounds are eluted using constant mobile phase composition. In **gradient elution** different compounds are eluted by increasing the strength of the organic solvent. The sample is injected while a weaker mobile phase is being applied to the system. The strength of the mobile phase is later increased in increments by raising the organic solvent fraction, which subsequently results in elution of retained components. This is usually done in a stepwise or linear fashion. **Polytyptic Mobile Phase**, sometimes referred to as mixed-mode chromatography, is a versatile method in which several types of chromatographic techniques, or modes, can be employed using the same column. These columns contain rigid macroporous hydrophobic resins covalently bonded to a hydrophilic organic layer. SEC, IEC, hydrophobic or affinity chromatography are some of the methods that may be utilized. By changing the the mobile phase, the mode of separation is thereby changed which allows the chromatographer to achieve the desired selectivity in the separations.

The **stationary phase** in HPLC refers to the solid support contained within the column over which the mobile phase continuously flows. The sample solution is injected into the mobile phase of the assay through the injector port. As the sample solution flows with the mobile phase through the stationary phase, the components of that solution will migrate according to the non-covalent interactions of the compounds with the stationary phase. The chemical interactions of the stationary phase and the sample with the mobile phase, determines the degree of migration and separation of the components contained in the sample. Columns containing various types of stationary phases are commercially available. Some of the more common stationary phases include: Liquid-Liquid, Liquid-Solid (Adsorption), Size Exclusion, Normal Phase, Reverse Phase, Ion Exchange, and Affinity.

Samples are injected into the HPLC via an injection port. The injection port of an HPLC commonly consists of an injection valve and the sample loop. The sample is typically dissolved in the mobile phase before injection into the sample loop. The sample is then drawn into a syringe and injected into the loop via the injection valve. A rotation of the valve rotor closes the valve and opens the loop in order to inject the sample into the stream of the mobile phase. Loop volumes can range between 10 µl to over 500 µl. In modern HPLC systems, the sample injection is typically automated.

There are several types of pumps available for use with HPLC analysis, they are: Reciprocating Piston Pumps, Syringe Type Pumps, and Constant Pressure Pumps.

The **detector** for an HPLC is the component that emits a response due to the eluting sample compound and subsequently signals a peak on the chromatogram. It is positioned immediately posterior to the stationary phase in order to detect the compounds as they elute from the column. The bandwidth and height of the peaks may usually be adjusted using the coarse and fine tuning controls, and the detection and sensitivity parameters may also be controlled (in most cases). There are many types of detectors that can be used with HPLC. Some of the more common detectors include: Refractive Index (RI), Ultra-Violet (UV), Fluorescent, Radiochemical, Electrochemical, Near-Infra Red (Near-IR), Mass Spectroscopy (MS), Nuclear Magnetic Resonance (NMR), and Light Scattering (LS).

Refractive Index (RI) detectors measure the ability of sample molecules to bend or refract light. This property for each molecule or compound is called its refractive index. For most RI detectors, light proceeds through a bi-modular flow-cell to a photodetector. One channel of the flow-cell directs the mobile phase passing through the column while the other directs only the mobile phase. Detection occurs when the light is bent due to samples eluting from the column, and this is read as a disparity between the two channels.

Ultra-Violet (UV) detectors measure the ability of a sample to absorb light. This can be accomplished at one or several wavelengths.

Fluorescent detectors measure the ability of a compound to absorb then re-emit light at given wavelengths. Each compound has a characteristic fluorescence. The excitation source passes through the flow-cell to a photodetector while a monochromator measures the emission wavelengths.

Mass Spectroscopy (MS) Detectors- The sample compound or molecule is ionized, it is passed through a mass analyzer, and the ion current is detected. There are various methods for ionization.

Nuclear Magnetic Resonance (NMR) Detectors- Certain nuclei with odd-numbered masses, including H and ^{13}C, spin about an axis in a random fashion. However, when placed between poles of a strong magnet, the spins are aligned either parallel or anti-parallel to the magnetic field, with the parallel orientation favored since it is slightly lower in energy. The nuclei are then irradiated with electromagnetic radiation which is absorbed and places the parallel nuclei into a higher energy state; consequently, they are now in "resonance" with the radiation. Each H or C will produce different spectra depending on their location and adjacent molecules, or elements in the compound, because all nuclei in molecules are surrounded by electron clouds which change the encompassing magnetic field and thereby alter the absorption frequency.

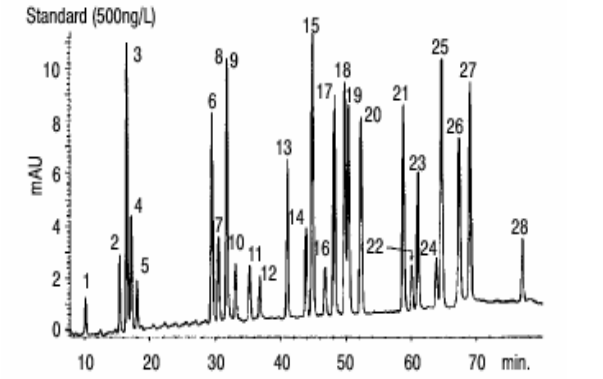
Light-Scattering (LS) Detectors- When a source emits a parallel beam of light which strikes particles in solution, some light is reflected, absorbed, transmitted, or scattered.

Near-Infrared Detectors- Operates by scanning compounds in a spectrum from 700 to 1100 nm. Stretching and bending vibrations of particular chemical bonds in each molecule are detected at certain wavelengths.

Liquid Chromatography Applications

Liquid chromatography has been used in an extremely wide range of analytical methods and it is impossible to give a comprehensive set of examples that would illustrate its wide applicability. The following are a few LC analyses that may indicate the scope of the technique and give the reader some idea of its importance and versatility.

An example of the use of reversed phase chromatography (employing a C18 column) for the separation of some pesticides is shown below. The column used was 25 cm long, 3.0 mm in diameter packed with silica based, C18 reverse phase packing particle size 5 µm. The mobile phase consisted of (A) 2 mM sodium acetate (pH 6.5) with 5% acetonitrile and (B) 100% acetonitrile and the flow-rate was 0.35 ml/min. The gradient was 2 min. 10% B; 10 to 70 min. 45% B. Column temperature was 40^0C and peaks detected by UV detector on 245 nm. The retention time of the last peak is about 80 minutes. This procedure trades efficiency for time and allows the separation to be achieved in the minimum time given the column and phase system that has been chosen.



1. Desisopropylatrazine
2. Metamitron
3. Fenuron
4. Chloridazon
5. Desethylatrazine
6. Metoxuron
7. Carbetamid
8. Bromacil
9. Hexazinon
10. Simazine
11. Metribuzin
12. Desethylterbutylazine
13. Carbutilat
14. Methabenzthiazuron
15. Chlortoluron
16. Atrazine
17. Monolinuron
18. Diuron
19. Isoproturon
20. Metobromuron
21. Metazachlor
22. Buturon
23. Propazine
24. Dimefuron
25. Terbuthylazine
26. Linuron
27. Chlorbromuron
28. Chloroxuron

Pesticides—Analysis of Pesticides in Drinking Water

Sample:	Pesticides
Column:	ZORBAX SB-C18, 3.0 x 250 mm, 5 µm, (P/N: 880975-302)
Mobile phase:	A = 2 mM Sodium Acetate (pH 6.5) with 5% ACN
	B = 100% Acetonitrile (ACN)
Gradient:	2 min, 10% B; 10 to 45% B in 70 min.
Flow rate:	0.35 mL/min
Temperature:	40°C
Detection:	UV 245 nm

E13. Gas Chromatograph

In gas chromatography (GC), the stationary phase is a high-boiling liquid and the mobile phase is an inert gas. The process of gas chromatography is carried out in a specially designed instrument. A very small amount of liquid mixture is injected into the instrument and is volatilized in a hot injection chamber. Then, it is swept by a stream of inert carrier gas through a heated column which contains the stationary, high-boiling liquid. As the mixture travels through this column, its components go back and forth at different rates between the gas phase and dissolution in the high-boiling liquid, and thus separate into pure components. Just before each compound exits the instrument, it passes through a detector. When the detector "sees" a compound, it sends an electronic message to the recorder, which responds by printing a peak on a piece of paper. Have a look at this schematic diagram of a gas chromatograph:

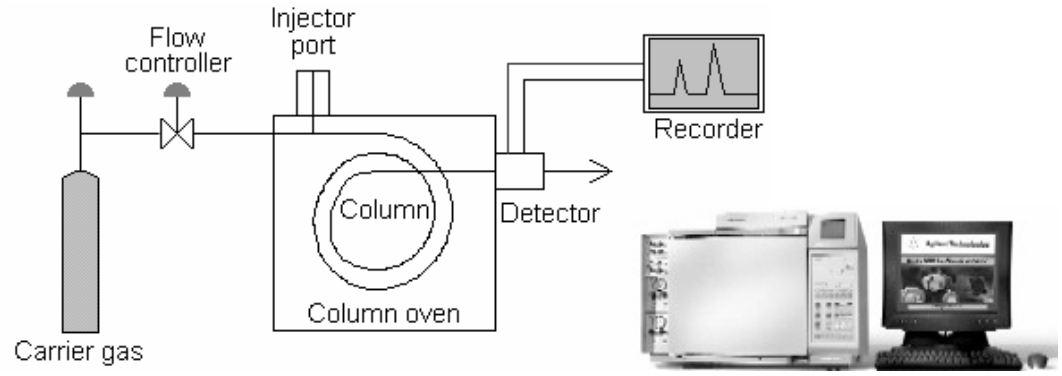


Fig. Gas Chromatograph and its flow diagram.

The GC consists of an injection block, a column, and a detector. An inert gas flows through the system. The injection chamber is a heated cavity which serves to volatilize the compounds. The sample is injected by syringe into this chamber through a port which is covered by a rubber septum. Once inside, the sample becomes vaporized and is carried out of the chamber and onto the column by the carrier gas.

The column is an integral part of the GC system. Inside the column is the important component: the stationary phase composed of the high-boiling liquid. There are two general types of column, ***packed* and *capillary*** (also known as *open tubular*). Packed columns contain a finely divided, inert, solid support material (commonly based on *diatomaceous earth*) coated with liquid stationary phase. Most packed columns are 1.5 - 10m in length and have an internal diameter of 2 - 4mm. Capillary columns have an internal diameter of a few tenths of a millimeter. They can be one of two types; *wall-coated open tubular* (WCOT) or *support-coated open tubular* (SCOT). Wall-coated columns consist of a capillary tube whose walls are coated with liquid stationary phase. In support-coated columns, the inner wall of the capillary is lined with a thin layer of

support material such as diatomaceous earth, onto which the stationary phase has been adsorbed. SCOT columns are generally less efficient than WCOT columns. Both types of capillary column are more efficient than packed columns.

The carrier gas is an inert gas, helium. The flow rate of the gas influences how fast a compound will travel through the column; the faster the flow rate, the lower the retention time. Generally, the flow rate is held constant throughout a run.

Each instrument will have a different setting for column temperature, injection port temperature, detector temperature.

There are many detectors which can be used in gas chromatography. Different detectors will give different types of selectivity. A *non-selective* detector responds to all compounds except the carrier gas, a *selective detector* responds to a range of compounds with a common physical or chemical property and a *specific detector* responds to a single chemical compound. Detectors can also be grouped into *concentration dependant detectors* and *mass flow dependant detectors*. The signal from a concentration dependant detector is related to the concentration of solute in the detector, and does not usually destroy the sample Dilution of with make-up gas will lower the detectors response. Mass flow dependant detectors usually destroy the sample, and the signal is related to the rate at which solute molecules enter the detector. The response of a mass flow dependant detector is unaffected by make-up gas. Have a look at this tabular summary of common GC detectors.

Two devices are used to record the GC traces/areas under peaks, integrating recorders and computer program. Each type of device records the messages sent to them by the detector as peaks, calculates the retention time, and calculates the area under each peak; all of this information is included in the printout. For similar compounds, the area under a GC peak is roughly proportional to the amount of compound injected.

Factors which affect GC separations

Efficient separation of compounds in GC is dependent on the compounds traveling through the column at different rates. The rate at which a compound travels through a particular GC system depends on the factors listed below:

1. *Volatility of compound:* Low boiling (volatile) components will travel faster through the column than will high boiling components .
2. *Polarity of compounds:* Polar compounds will move more slowly, especially if the column is polar.
3. *Column temperature:* Raising the column temperature speeds up all the compounds in a mixture.

4. *Column packing polarity:* Usually, all compounds will move slower on polar columns, but polar compounds will show a larger effect.
5. *Flow rate of the gas* through the column: Speeding up the carrier gas flow increases the speed with which all compounds move through the column.
6. *Length of the column:* The longer the column, the longer it will take all compounds to elute. Longer columns are employed to obtain better separation.

Generally the number one factor to consider in separation of compounds on the GCs in the teaching labs is the **boiling points of the different components**. Differences in polarity of the compounds are only important if you are separating a mixture of compounds which have widely different polarities. Column temperature, the polarity of the column, flow rate, and length of a column are constant in GC runs in the Organic Chemistry Teaching Labs. For each planned GC experiment, these factors have been optimized to separate your compounds and the instrument set up by the staff.

E14. Dissolved oxygen meter

There are two types of DO probes available, including a laboratory probe and a field probe. Both types operate on the same principle. An advantage of the field probe is that it eliminates the need to collect, transport and then performs analysis on a sample.

Oxygen-sensitive membrane probes contain two solid metal electrodes immersed in an electrolyte solution which allows the passage of an electrical current. The electrode pair is covered with a semi-permeable membrane which allows the passage of DO molecules. When the oxygen molecules pass through the membrane, they cause the electrical current already present to change. This change is reflected by the movement of the needle on the meter.

Allow the instrument to warm up for at least 15 minutes before taking the first reading of the day. Leave the instrument on between measurements. This will eliminate the need for warm-up periods between tests. Calibrate at least once per day. Distilled or deionized water, and not effluent, should be used for the probe calibration. Always stir the sample while taking a reading.

APPENDIX – 1

SPECIFIC INTERFERENCE PROBLEMS IN ELEMENTAL ANALYSIS BY AAS

Aluminum: Aluminum may be as much as 15% ionized in a nitrous oxide/acetylene flame. Use of an ionization suppressor (1000 µg/ml K as KCl) will eliminate this interference.

Antimony: In the presence of lead (1,000 mg/l), a spectral interference may occur at the 217.6-nm resonance line. In this case, the 231.1-nm resonance line should be used. Excess concentrations of copper and nickel (and potentially other elements), as well as acids, can interfere with antimony analyses. If the sample contains these matrix types, either matrices of the standards should be matched to those of the sample or the sample should be analyzed using a nitrous oxide/acetylene flame.

Barium: Barium undergoes significant ionization in the nitrous oxide/acetylene flame, resulting in a significant decrease in sensitivity. All samples and standards must contain 2 ml of the KCl ionization suppressant per 100 ml of solution. In addition, high hollow cathode current settings and a narrow spectral band pass must be used because both barium and calcium emit strongly at barium's analytical wavelength.

Beryllium: Concentrations of Al greater than 500 ppm may suppress beryllium absorbance. The addition of 0.1% fluoride has been found effective in eliminating this interference. High concentrations of magnesium and silicon cause similar problems and require the use of the method of standard additions.

Calcium: All elements forming stable oxyanions will complex calcium and interfere unless lanthanum is added. Addition of lanthanum (0.1 – 1 %) to prepared samples rarely presents a problem because virtually all environmental samples contain sufficient calcium to require dilution to be within the linear range of the method.

Chromium: Ionization interference may occur if the samples have significantly higher alkali metal content than the standards. If this interference is encountered, an ionization suppressant (KCl) should be added to both samples and standards.

Magnesium: All elements forming stable oxyanions (P, B, Si, Cr, S, V, Ti, Al, etc.) will complex magnesium and interfere unless lanthanum is added. Addition of lanthanum to prepared samples rarely presents a problem because virtually all environmental samples contain sufficient magnesium to require dilution.

Molybdenum: Interference in an air/acetylene flame from Ca, Sr, SO_4, and Fe are severe. These interferences are greatly reduced in the nitrous oxide flame and by the addition of 1000 mg/l aluminum to samples and standards.

Nickel: High concentrations of iron, cobalt, or chromium may interfere, requiring either matrix matching or use of a nitrous-oxide/acetylene flame. A non-response line of Ni at 232.14 nm causes non-linear calibration curves at moderate to high nickel concentrations, requiring sample dilution or use of the 352.4 nm line.

Osmium: Due to the volatility of osmium, standards must be made on a daily basis, and the applicability of sample preparation techniques must be verified for the sample matrices of interest.

Potassium: In air/acetylene or other high temperature flames (>2800^{o}C), potassium can experience partial ionization, which indirectly affects absorption sensitivity. The presence of other alkali salts in the sample can reduce ionization and thereby enhance analytical results. The ionization-suppressive effect of sodium is small if the ratio of Na to K is under 10. Any enhancement due to sodium can be stabilized by adding excess sodium (1000 µg/ml) to both sample and standard solutions. If more stringent control of ionization is required, the addition of cesium should be considered.

Silver: Since silver nitrate solutions are light sensitive and have the tendency to plate silver out on the container walls, they should be stored in dark-colored bottles. In addition, it is recommended that the stock standard concentrations be kept below 2 ppm and the chloride content increased to prevent precipitation. If precipitation is occurring, a 5%:2% HCl : HNO_3 stock solution may prevent precipitation. Daily standard preparation may also be needed to prevent precipitation of silver.

Strontium: Chemical interference caused by silicon, aluminum, and phosphate are controlled by adding lanthanum chloride. Potassium chloride is added to suppress the ionization of strontium. All samples and standards should contain 1 ml of lanthanum chloride/potassium chloride solution per 10 ml of solution.

Vanadium: High concentrations of aluminum or titanium, or the presence of Bi, Cr, Fe, acetic acid, phosphoric acid, surfactants, detergents, or alkali metals, may interfere. The interference can be controlled by adding 1,000 mg/L aluminum to samples and standards.

Zinc: High levels of silicon, copper, or phosphate may interfere. Addition of strontium (1,500 mg/l) removes the copper and phosphate interference.

APPENDIX - 2

PREPARATION OF STANDARD STOCK SOLUTION

Aluminum: Dissolve 1.000 g of aluminum metal in dilute HCl with gentle warming and dilute to 1 liter with reagent water.

Antimony: Carefully weigh 2.743 g of antimony potassium tartrate, $K(SbO)C_4H_4O_6C1/2H_2O$, and dissolve in reagent water. Dilute to 1 liter with reagent water.

Barium: Dissolve 1.779 g barium chloride, $BaCl_2.2H_2O$, analytical grade and dilute to 1 liter with reagent water.

Beryllium: Dissolve 11.659 g beryllium sulfate, $BeSO_4$, in reagent water containing 2 mL nitric acid (conc.) and dilute to 1 liter with reagent water.

Cadmium: Dissolve 1.000 g cadmium metal in 20 ml of 1:1 HNO_3 and dilute to 1 liter with reagent water.

Calcium: Suspend 2.500 g of calcium carbonate, $CaCO_3$, dried for 1 hour at 180^oC in reagent water and dissolve by adding a minimum of dilute HCl. Dilute to 1 liter with reagent water.

Chromium: Dissolve 1.923 g of chromium trioxide, CrO_3, in reagent water, acidify (to pH # 2) with redistilled HNO_3 (conc.), and dilute to 1 liter with reagent water.

Cobalt: Dissolve 1.000 g of cobalt metal in 20 ml of 1:1 HNO_3 and dilute to 1 liter with reagent water. Chloride or nitrate salts of cobalt (II) may be used. Although numerous hydrated forms exist, they are not recommended unless the exact composition of the compound is known.

Copper: Dissolve 1.000 g of electrolytic copper in 5 ml of redistilled HNO_3 (conc.) and dilute to 1 liter with reagent water.

Iron: Dissolve 1.000 g iron wire in 10 ml redistilled HNO_3 (conc.) and reagent water and dilute to 1 liter with reagent water. Note that iron passivates in conc. HNO_3, and therefore some water should be present.

Lead: Dissolve 1.599 g of lead nitrate, $Pb(NO_3)_2$, in reagent water, acidify with 10 ml redistilled HNO_3 (conc.), and dilute to 1 liter with reagent water.

Lithium: Dissolve 5.324 g lithium carbonate, Li_2CO_3, in a minimum volume of 1:1 HCl and dilute to 1 liter with reagent water.

Magnesium: Dissolve 1.000 g of magnesium metal in 20 ml 1:1 HNO_3 and dilute to 1 liter with reagent water.

Manganese: Dissolve 1.000 g manganese metal in 10 ml redistilled HNO_3 (conc.) and dilute to 1 liter with reagent water.

Molybdenum: Dissolve 1.840 g of ammonium molybdate, $(NH_4)_6Mo_7O_{24}.H_2O$, and dilute to 1 liter with reagent water.

Nickel: Dissolve 1.000 g nickel metal or 4.953 g nickel nitrate, Ni $(NO_3)_2.6H_2O$, in 10 ml HNO_3 (conc.) and dilute to 1 liter with reagent water.

Osmium: Procure a certified aqueous standard from a supplier and verify by comparison with a second standard. If necessary, standards can be made from osmium compounds. However, due to the toxicity of these compounds, this approach is not advised.

Potassium: Dissolve 1.907 g of potassium chloride, KCl , dried at 110^oC, in reagent water and dilute to 1 liter with reagent water.

Silver: Dissolve 1.575 g of anhydrous silver nitrate, $AgNO_3$, in reagent water. Add 10 mL of HNO_3 (conc.) and dilute to 1 liter with reagent water. Store in a dark-colored glass bottle in a refrigerator.

Sodium: Dissolve 2.542 g sodium chloride, NaCl, in reagent water, acidify with 10 ml redistilled HNO_3 (conc.), and dilute to 1 liter with reagent water.

Strontium: Dissolve 2.415 g of strontium nitrate, $Sr(NO_3)_2$, in 10 ml of conc. HCl and 700 ml of reagent water. Dilute to 1 liter with reagent water.

Thallium: Dissolve 1.303 g thallium nitrate, $TlNO_3$, in reagent water, acidify (to pH # 2) with 10 ml conc. HNO_3, and dilute to 1 liter with reagent water.

Tin: Dissolve 1.000 g of tin metal in 100 ml conc. HCl and dilute to 1 liter with reagent water.

Vanadium: Dissolve 1.785 g of vanadium pentaoxide, V_2O_5 , in 10 ml of conc. HNO_3 and dilute to 1 liter with reagent water.

Zinc: Dissolve 1.000 g zinc metal in 10 ml of conc. HNO_3 and dilute to 1liter with reagent water.

APPENDIX - 3

INSTRUMENT SETTING FOR ATOMIC ABSORPTION SPECTROPHOTOMETERY

Element	Wave length (nm)	Slit width (nm)	Working range (µg / ml)	Sensitivity (µg / ml)	Lamp current (mA)	Flame type
Al	309.3	0.5	25-135	1.0	25.0	N_2O-C_2H_2
Al	396.2	0.5	25-110	1.0	25.0	N_2O-C_2H_2
As	193.7	0.7	1-100	45.0	300.0	MHS
Ca	422.7	0.5	1-4	0.02	10.0	N_2O-C_2H_2
Ca	422.7	0.5	1-10	0.09	10.0	Air-C_2H_2
Cd	228.8	0.5	0.5-5	0.03	15.0	Air-C_2H_2
Co	240.7	0.2	1-20	0.2	6.0	Air-C_2H_2
Cr	357.9	0.2	2-20	0.2	25.0	Air-C_2H_2
Cr	425.4	0.2	7-40	0.5	25.0	Air-C_2H_2
Cu	324.7	0.5	1-20	0.1	15.0	Air-C_2H_2
Cu	217.9	0.2	7.5-30	0.2	15.0	Air-C_2H_2
Fe	248.3	0.2	2-20	0.1	30.0	Air-C_2H_2
Fe	372.0	0.2	20-80	0.5	30.0	Air-C_2H_2
Hg	253.7	0.7	1-200	4.2	150.0	MHS
K	766.5	0.5	1-10	0.01	6.0	Air-C_2H_2
Mg	285.2	0.5	0.1-2	0.01	6.0	Air-C_2H_2
Mg	202.6	1.0	5-20	0.1	6.0	Air-C_2H_2
Mn	279.5	0.2	1-10	0.06	5.0	Air-C_2H_2
Mn	403.1	0.2	7-27	0.2	5.0	Air-C_2H_2
Mo	313.3	0.2	10-50	0.8	7.0	N_2O-C_2H_2
Na	589.0	0.2	0.03-1	0.02	8.0	Air-C_2H_2
Ni	232.0	0.2	2-20	0.2	25.0	Air-C_2H_2
Ni	352.4	0.5	6-30	0.2	25.0	Air-C_2H_2
Pb	283.3	0.5	4-40	0.2	440.0	Air-C_2H_2
Se	196.1	1.0	45-180	1.0	10.0	N_2O-C_2H_2
Si	251.6	0.2	20-200	2.0	15.0	N_2O-C_2H_2
Zn	213.9	0.5	0.5-5	0.03	15.0	Air-C_2H_2

APPENDIX - 4

INDIAN STANDARD DRINKING WATER - SPECIFICATION (BIS 10500: 1991)

Sl.No	Substance or Characteristic	Requirement (Desirable Limit)	Permissible Limit in the absence of alternate source
1.	Colour, (Hazen units)	5	25
2.	Odour	Unobjectonable	Unobjectionable
3.	Taste	Agreeable	Agreeable
4.	Turbidity (NTU)	5	10
5.	pH Value	6.5 to 8.5	No Relaxation
6.	Total Hardness (as $CaCo_3$) mg/l	300	600
7.	Iron (as Fe) mg/l	0.3	1.0
8.	Chlorides (as Cl) mg/l	250	1000
9.	Residual, free chlorine, mg /l	0.2	--
10.	Dissolved solids mg/l	500	2000
11.	Calcium (as Ca) mg/l	75	200
12.	Copper (as Cu) mg/l	0.05	1.5
13	Manganese (as Mn) mg/l	0.10	0.3
14	Sulfate (as SO_4) mg/l	200	400
15	Nitrate (as NO_3) mg/l	45	100
16	Fluoride (as F) mg/l	1.9	1.5
17	Phenolic compounds mg/l	0.001	0.002
18	Mercury (as Hg) mg/l	0.001	No relaxation
19	Cadmiun (as Cd) mg/l	0.01	No relaxation
20	Selenium (as Se) mg/l	0.01	No relaxation
21	Arsenic (as As) mg/l	0.05	No relaxation
22	Cyanide (as CN) mg/l	0.05	No relaxation
23	Lead (as Pb) mg/l	0.05	No relaxation
24	Zinc (as Zn) mg/l	5	15
25	Anionic detergents (as MBAS) mg/l	0.2	1.0
26	Chromium (as Cr^{6+}) mg/l	0.05	No relaxation
27	Polynuclear aromatic hydro carbons (as PAH) g/l	--	--
28	Mineral Oil mg/l	0.01	0.03
29	Pesticides mg/l	Absent	0.001
30	Radioactive Materials		
	i. Alpha emitters Bq/l	--	0.1
	ii. Beta emitters pci/l	--	1.0
31	Alkalinity mg/l	200	600
32	Aluminium (as Al) mg/l	0.03	0.2
33	Boron mg/l	1	5

APPENDIX - 5

MOLARITIES AND NORMALITY OF COMMON ACIDS AND BASE

Commodity	Specific Gravity	Molarity (Normality)	Volume (ml) of acid/alkali required for 1 lit of 1 N solution
Acetic acid, glacial	1.049	17.5 (17.0)	57.0
Formic acid, 98% min	1.218	25.9	38.0
Hydrochloric acid, 35-38%	1.118	11.8 (12.0)	86.0
Nitric acid, 69% min	1.42	15.4 (15.0)	64.0
Orthophosphoric acid, 85%	1.75	14.6 (44.0)	23.0
Perchloric acid, 60% min	1.54	9.2	109.0
Perchloric acid, 70%	1.70	11.6	86.0
Sulphuric acid, 98% min	1.84	18.3 (36.7)	27.0
Ammonia solution, 35% min	0.80	18.1 (15.0)	56.0
Potassium hydroxide	-	-	56.0 g
Sodium hydroxide	-	-	40.0 g