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GUIDE MANUAL : WATER AND WASTE WATER ANALYSIS

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CENTRAL POLLUTION CONTROL BOARD
(Ministry of Environment and Forest)
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Website:www.cpcb.nic.in
February 2012

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on behalf of Sh. J. S. Kamyotra, Member Secretary, CPCB

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FOREWORD

Central Pollution Control Board is entrusted for surface water and groundwater monitoring in Hydrology Project-II implemented by Ministry of Water Resources (MoWR) with assistance from the World Bank (IBRD) along with other agencies in thirteen states (Andhra Pradesh, Chhattisgarh, Gujarat, Kerala, Karnataka, Madhya Pradesh, Maharashtra, Orissa, Tamil Nadu, Himachal Pradesh, Goa, Pondicherry and Punjab); and eight central agencies CWC, CGWB, NIH, CWPRS, IMD, BBMB, including MoWR.

The project development objective is to extend and promote the effective use of the Hydrologic Information System (HIS) by all potential stakeholders concerned with water resources planning and management, both public and private. This may improve the productivity and cost - effectiveness of water-related investments. CPCB extends technical support in laboratory development & preparation of specifications of water quality equipment for laboratories at various levels.

This document "Guide Manual: Water and Wastewater Analysis" is to cater the need of evolving a simplified code of practice for the laboratories engaged in carrying out water quality assessment under Hydrology Project -II. Development and proper use of such a manual by concerned laboratories brings homogeneity for ensuring quality assurance especially in water and wastewater analysis.

Hopefully, the information and guidelines contained here will provide necessary assistance to the HP-II Laboratories, State Pollution Control Boards and other bodies engaged in water and wastewater analysis.

The Manual has been prepared by Dr. Reeta Kori, Expert Resource Person, Mrs. Suniti Parashar, SSA, under the guidance of Dr. D.D. Basu, Scientist 'E' & Sh. J.S.Kamyotra, Member Secretary, CPCB. Ms. Sarita Kumari, DEO assisted in the typing of the manual.

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1 INTRODUCTION TO THE GUIDANCE MANUAL

1.1. Collection and Preservation of Samples

The objective of sampling is to collect representative sample. Representative sample means a sample in which relative proportions or concentration of all pertinent components will be the same as in the material being sampled. Moreover, the same sample will be handled in such a way that no significant changes in composition occur before the tests are made. The sample volume shall optimal small enough that it can be transported and large enough for analytical purposes.

Because of the increasing pressure placed on verifying the accuracy and representatives of data, greater emphasis is placed on proper sample collection, tracking, and preservation techniques. Often laboratory personnel help in planning a sampling program, in consultation with the user of the test results. Such consultation is essential to ensure selecting samples and analytical methods that provide a sound and valid basis for answering the questions that prompted the sampling and that will meet regulatory and/or project-specific requirements.

This section addresses the collection and preservation of water and wastewater samples; the general principles also apply to the sampling of solids or semisolid matrices.

1.1.1 General Requirements

- Obtain a sample that meets the requirements of the sampling program and handle it so that it does not deteriorate or become contaminated before it is analyzed.

- Ensure that all sampling equipments are clean and quality-assured before use. Use sample containers that are clean and free of contaminants. Bake at 450°C all bottles to be used for organic analysis sampling.

- Fill sample containers without prerinsing with sample; prerinsing results in loss of any pre-added preservative and sometimes can bias results high when certain components adhere to the sides of the container. Depending on determinations to be performed, fill the container full (most organic compound determinations) or leave space for aeration, mixing, etc. (microbiological and inorganic analyses). If the bottle already contains preservative, take care not to overfill the bottle, as preservative may be lost or diluted. Except when sampling for analysis of volatile organic compounds, leave an air space equivalent to approximately 1% of the container volume to allow for thermal expansion during shipment.

- Special precautions (discussed below) are necessary for samples containing organic compounds and trace metals. Since many constituents may be present at low concentrations (micro-grams or nanograms per liter), they may be totally or partially lost or easily contaminated when proper sampling and preservation procedures are not followed.

- Composite samples can be obtained by collecting over a period of time, or at many different over a period of time, depth, or at many different sampling points. The details of collection vary with local conditions, so specific recommendations are not universally applicable. Sometimes it is more informative to analyze numerous separate samples instead of one composite so that variability, maxima, and minima can be determined.

- Because of the inherent instability of certain properties and compounds, composite sampling for some analytes is not recommended where quantitative values are desired (examples in-residual, iodine, hexavalent chromium, nitrate, volatile organic compounds, radon-222, dissolved oxygen, ozone, temperature, and pH). In certain cases, such as for BOD, composite samples are collected routinely by regulatory agencies. Refrigerate composite samples for BOD and nitrite.

- Important factors affecting results are the presence of suspended matter or turbidity, the method chosen for removing a sample from its container, and the physical and chemical brought about by storage or aeration. Detailed procedures are essential when processing (blending, sieving, filtering) samples to be analyzed for trace constituents, especially metals and organic compounds. Some determinations can be invalidated by contamination during processing. Treat each sample individually with regard to the substances to be determined, the amount and nature of turbidity present, and other conditions that may influence the results.

- For metals it is often appropriate to collect both filtered and unfiltered sample to differentiate between total and dissolved metals present in the matrix. Be aware that some metals may partially absorb to filters. Beforehand, determine the acid requirements to bring the pH to <2 on a separate sample. Add the same relative amount of acid to all samples; use ultrapure acid preservative to prevent contamination. When filtered samples are to be collected, filter them, if possible, in the field, or at the point of collection before preservation with acid. Filter samples in a laboratory-controlled environment if field conditions could cause error or contamination; in this case filter as soon as possible. Often slight turbidity can be tolerated if experience shows that it will cause no interference in gravimetric or volumetric tests and that its influence can be corrected in colorimetric tests, where it has potentially the greatest interfering effect. Sample collector must state whether or not the sample has been filtered.

- Record of sample shall be as follows:

General information

- Sample identification number
- Location
- Sample collector
- Date and hour
- Sample type (Grab or composite)

Specific information

- Water temperature
- Weather
- Stream blow
- Water level
- Any other information

The information may be attached tag, labeling or writing on container with water proof ink.

- Description of sampling points
    - By map using landmarks
    - Use global positioning system

- When samples are collected from a river or stream, observed results may vary with depth, stream flow, and distance from each shore. Selection of the number and distribution of sites at which samples should be collected depends on study objectives, stream characteristics, available equipment, and other factors. If equipment is available, take an integrated sample form top to bottom in the middle of the main channel of the stream or from side to side at mid-depth. If only grab or catch samples can be collected, preferably take them at various points of equal distance across the steam; if only one sample can be collected, take it in the middle of the main channel of the stream and at mid-depth.

- Rivers, streams, lakes, and reservoirs are subject to considerable variations from normal causes such as seasonal stratification, diurnal variations, rainfall, runoff, and wind. Choose location, depth, and frequency of sampling depending on local conditions and the purpose of the investigation. Avoid areas of excessive turbulence because of potential loss of volatile constituents and of potential presence of denser-than-air-toxic vapors. Avoid sampling at weirs if possible because such locations tend to favor retrieval of lighter-than-water, immiscible compounds. Generally, collect samples beneath the surface in quiescent areas and open sampling container below surface with the mouth directed toward the current to avoid collecting surface scum unless oil and grease is a constituent of interest; then collect water at the surface. If composite samples are required, ensure that sample constituents are not lost during compositing because of improper handling of portions being composited. If samples will be analyzed for organic constituents, refrigerate composited portions.

Do not composite samples for VOC analysis because some of the components will be lost through volatilization.

1.1.2 Safety Considerations

- Always prohibit eating, drinking, or smoking near samples, sampling locations, and in the laboratory. Keep sparks, flames, and excessive heat sources away from samples and sampling locations. If flammable compounds are suspected of known to be present and samples are to be refrigerated, use only specially designed *explosion-proof* refrigerators[2].

- Label adequately any sample known or suspected to be hazardous because of flammability, corrosivity, toxicity, oxidizing chemicals, or radioactivity, so that appropriate precautions can be taken during sample handling, storage, and disposal.

1.1.3 References

1. FORSBERG, K. & L.H. KEITH. 1988. Instant Gloves and CPC Database, Instant Reference Sources, Inc. Austin, Tex.
2. WATER POLLUTION CONTROL FEDERATION. 1986. Removal of Hazardous Wastes in Wastewater Facilities-Halogenated Organics. Manual of Practices FD-11, Water Pollution Control Fed., Alex-andria, Va.

1.2 Collection of Samples

1.2.1 Types of samples

a. *Grab samples:* Grab samples are single collected at a specific spot at a site over a short period of time (typically seconds or minutes). Thus, they represent a "snapshot" in both space and time of a sampling area. Discrete grab samples are taken at a selected location, depth, and time. Depth-integrated grab samples are collected over a predetermined part of the entire depth of a water column, at a selected location and time in a given body of water.

A sample can represent only the composition of its source at the time and place of collection. However, when a source is known to be relatively constant in composition over an extended time or over substantial distances in all directions, then the sample may represent a longer time period and/or a larger volume than the specific time and place at which it was collected. In such circumstances, a source may be represented adequately by single grab samples. Examples are protected groundwater supplies, water supplies receiving conventional treatment, some well-mixed surface waters, but rarely, wastewater streams, rivers, large lakes, shorelines, estuaries, and groundwater plumes.

When a source is known to vary with time, grab samples collected at suitable intervals and analyzed separately can document the extent, frequency, and duration of these variations. Choose sampling intervals on the basis of the expected frequency of changes, which may vary from as little as 5 min to as long as 1h or more. Seasonal variations in natural systems may necessitate sampling over months. When the source composition varies in space (i.e. from location to location) rather than time, collect samples from appropriate locations that will meet the objectives of the study (for example, upstream and downstream from a point source, etc.).

b. *Composite samples:* Composite samples should provide a more representative sampling of heterogeneous matrices in which the concentration of the analytes of interest may vary over short periods of time and/or space. Composite samples can be obtained by combining portions of multiple grab samples or by using specially designed automatic sampling devices. Sequential (time) composite samples are collected by using continuous, constant sample pumping or by mixing equal water volumes collected at regular time intervals. Flow-proportional composites are collected by continuous pumping at a rate proportional to the flow, by mixing equal volumes of water collected at time intervals that are inversely proportional to the volume of flow, or by mixing volumes of water proportional to the flow collected during or at regular time intervals.

Advantages of composite samples include reduced costs of analyzing a large number of samples, more representative samples of heterogeneous matrices, and larger sample sizes when amounts of test samples are limited. Disadvantages of composite samples include loss of analyte relationships in individual samples, potential dilution of analytes below detection levels, increased potential analytical interferences, and increased possibility of analyte interactions. In addition, use of composite samples may reduce the number of samples analyzed below the required statistical need for specified data quality objectives or project-specific objectives.

Do not use composite samples with components or characteristics subject to significant and unavoidable changes during storage. Analyze individual samples as soon as possible after collection and preferably at the sampling point. Examples are dissolved gases, residual chlorine, soluble sulfide, temperature, and pH. Changes in components such as dissolved oxygen or carbon dioxide, pH, or temperature may produce secondary changes in certain inorganic constituents such as iron, manganese, alkalinity, or hardness. Some organic analytes also may be changed by changes in the foregoing components. Use time-composite samples only for determining components that can be demonstrated to remain unchanged under the conditions of sample collection, preservation, and storage.

Collect individual portions in a wide-mouth bottle every hour (in some cases every half hour or even every 5 min) and mix at the end of the sampling period or combine in a single bottle as collected. If preservatives are used, add them to the sample bottle initially so that all portions of the composite are preserved as soon as collected.

Automatic sampling devices are available; however, do not use them unless the sample is preserved as described below. Composite samplers running for extended periods (week to months) should undergo routine cleaning of containers and sample lines to minimize sample growth and deposits.

c. *Integrated (discharge*-weighted) samples: For certain purposes, the information needed is best provided by analyzing mixtures of grab samples collected from different points simultaneously, or as nearly so as possible, using discharge-weighted methods such as equal-width increment (EWI) or equal discharge-increment (EDI) procedures and equipment. An example of the need for integrated sampling occurs in a river or stream that varies in composition across its width and depth. To evaluate average composition or total loading, use a mixture of samples representing various points in the cross-section, in proportion to their relative flows. The need for integrated samples also may exist if combined treatment is proposed for several separate wastewater streams, the interaction of which may have a significant effect on treatability or even on composition. Mathematical prediction of the interactions among chemical components may be inaccurate or impossible and testing a suitable integrated sample may provide useful information.

Both lakes and reservoirs show spatial variations of composition (depth and horizontal location). However, there are conditions under which neither total nor average results are especially useful, but local variations are more important. In such cases, examine samples separately (i.e., do not integrate them).

Preparation of integrated samples usually requires equipment designed to collect a sample water uniformly across the depth profile. Knowledge of the volume, movement, and composition of the various parts of the water being sampled usually is required. Collecting integrated samples is a complicated and specialized process that must be described in a sampling plan.

1.2.2 Chain-of-Custody Procedures

Properly designed and executed chain-of-custody forms will ensure sample integrity from collection to data reporting. This includes the ability to trace possession and handling of the sample from the time of collection through analysis and final disposition. This process is referred to as "chain-of-custody" and is required to demonstrate sample control when the data are to be used for regulation or litigation. Where litigation is not involved, chain-of-custody procedures are useful for routine control of samples.

A sample is considered to be under a person's custody if it is in the individual's physical possession, in the individual's sight, secured and tamper-proofed by that individual, or secured in an area restricted to authorized personnel. The following procedures summarize the major aspects of chain-of-custody. More detailed discussions are available.[1,2]

a. *Sample labels (including bar-code labels):* Use labels to prevent sample misidentification. Gummed paper labels or tags generally are adequate. Include at

least the following information: a unique sample number, sample type, name of collector, date and time of collection, place of collection, and sample preservative. Also include date and time of preservation for comparison to date and time of collection. Affix tags or self-adhesive labels to sample containers before, or at the time of, sample collection.

b. *Sample seals:* Use sample seals to detect unauthorized tampering with samples up to the time of analysis. Use self-adhesive paper seals that includes at least the following information: sample number (identical with number on sample label), collector's name, and date and time of sampling. Plastic shrink seals also may be used.

   Attach seal in such a way that it is necessary to break it to open the sample container or the sample shipping container (e.g., a cooler). Affix seal to container before sample leaves custody of sampling personnel.

c. *Field log book:* Record all information pertinent to a field survey or sampling in a bound log book. As a minimum, include the following in the log book: purpose of sampling; location of sampling point; name and address of field contact; producer of material being sampled and address, if different from location; type of sample; and method, date, and time of preservation. If the sample is wastewater, identify process producing waste stream. Also provide suspected sample composition, including concentrations; number and volume of sample(s) taken; description of sampling point and sampling method; date and time of collection; collector's sample identification number(s); sample distribution and how transported; reference such as maps or photographs of the sampling site; field observations and measurements; and signatures of personnel responsible for observations. Because sampling situations vary widely, it is essential to record sufficient information so that one could reconstruct the sampling event without reliance on the collector's memory. Protect log book and keep it in a safe place.

d. *Chain-of custody record:* Fill out a chain-of-custody record to accompany each sample or group of samples. The record includes the following information: sample number; signature of collector; date, time, and address of collection; sample type; sample preservation requirements; signatures of persons involved in the chain of possession; and inclusive dates and times of possession.

e. *Sample analysis request sheet:* The sample analysis request sheet accompanies samples to the laboratory. The collector completes the field portion of such a form that includes most of the pertinent information noted in the log book. The laboratory portion of such a form is to be completed by laboratory personnel and includes: name of person receiving the sample, laboratory sample number, date of sample receipt, condition of each sample (i.e., if it is cold or warm, whether the container is full or not, color, if more than one phase is present, etc.) and determinations to be performed.

f. *Sample delivery to the laboratory:* Deliver sample(s) to laboratory as soon as

practicable after collection, typically within 2 d. Where shorter sample holding times are required, make special arrangements to insure timely delivery to the laboratory. Where samples are shipped by a commercial carrier, include the waybill number in the sample custody documentation. Ensure that samples are accompanied by a complete chain-of-custody record and a sample analysis request sheet. Deliver sample to sample custodian.

g. *Receipt and logging of sample:* In the laboratory, the sample custodian inspects the condition and seal of the sample and reconciles label information and seal against the chain-of-custody record before the sample is accepted for analysis. After acceptance, the custodian assigns a laboratory number, logs sample in the laboratory log book and/or computerized laboratory information management system, and stores it in a secured storage room or cabinet or refrigerator at the specified temperature until it is assigned to an analyst.

h. *Assignment of sample for analysis:* The laboratory supervisor usually assigns the sample for analysis. Once the sample is in the laboratory, the supervisor or analyst is responsible for its care and custody.

i. *Disposal:* Hold samples for the prescribed amount of time for the project or until the data have been reviewed and accepted. Document the disposition of samples. Ensure that disposal is in accordance with local, state, and U.S. EPA approved methods.

1.2.3 Sampling Methods

a. *Manual sampling:* Manual sampling involves minimal equipment but may be unduly costly and time-consuming for routine or large-scale sampling programs. It requires trained field technicians and is often necessary for regulatory and research investigations for which critical appraisal of field conditions and complex sample collection techniques are essential. Manually collect certain samples, such as waters containing oil and grease.

b. *Automatic sampling:* Automatic samplers can eliminate human errors in manual sampling, can reduce labor costs, may provide the means for more frequent sampling,[3] and are used increasingly. Be sure that the automatic sampler does not contaminate the sample. For example, plastic components may be incompatible with certain organic compounds that are soluble in the plastic parts or that can contaminated (e.g., from phthalate esters) by contact with them. If sample constituents are generally known, contact the manufacturer of an automatic sampler regarding potential incompatibility of plastic components.

   Program an automatic sampler in accordance with sampling needs. Carefully match pump speeds and tubing sizes to the type of sample to be taken.

c. *Sorbent sampling:* Use of solid sorbents, particularly membrane-type disks, is becoming more frequent. These methods offer advantages of rapid, inexpensive

sampling if the analytes of interest can be adsorbed and desorbed efficiently and the water matrix is free of particulates that plug the sorbent.

1.2.4 Sample Containers

The type of sample containers used is of utmost importance. Test sample containers and document that they are free of analytes of interest, especially when sampling and analyzing for very low analyte levels. Containers typically are made of plastic or glass, but one material may be preferred over the other. For example, silica, sodium, and boron may be leached from soft glass but not plastic, and trace levels of same pesticides and metals may sorb onto the walls of glass containers.[4] Thus, hard glass containers* are preferred. For samples containing organic compounds, do not use plastic containers except those made of fluorinated polymers such as polytetrafluoroethylene (PTFE).[3]

Some sample analytes may dissolve (be absorbed) into the walls of plastic containers; similarly, contaminants from plastic containers may leach into samples. Avoid plastics wherever possible because of potential contamination from phthalate esters. Containers failure due to breakdown of the plastic is possible. Therefore, use glass containers for all organics analyses such as volatile organics, semivolatile organics, pesticides, PCBs, oil and grease. Some analytes (e.g., bromine-containing compounds and some pesticides. Polynuclear aromatic compounds, etc.) are light sensitive; collect them in amber-colored glass containers to minimize photodegradation. Container caps, typically plastic, also can be a problem. Do not use caps with paper liners. Use foil or PTFE liners but be aware that metal liners can contaminate samples collected for metals analysis and they may also react with the sample if it is acidic or alkaline. Serum vials with PTFE-lined rubber or plastic septa are useful.

In rare situations it may be necessary to use sample containers not specifically prepared for use, or otherwise unsuitable for the particular situation; thoroughly document these deviations. Documentations should include type and source of container, and the preparation technique, e.g., acid washed with reagent water rinse. For QA purposes the inclusion of a bottle blank may be necessary.

1.2.5 Number of Samples

Because of variability from analytical and sampling procedures (i.e., population variability), a single sample is insufficient to reach any reasonable desired level of confidence. If an overall standard deviation (i.e. the standard deviation of combined sampling and analysis) is known, the required number of samples for a mobile matrix such as water may be estimated as follows.[4]

$$N \geq \{(ts / U)^2$$

Where:
N = number of samples.

t = Student-t statistic for a given confidence level,
s = overall standard deviation, and
U = acceptable level of uncertainty.

To assist in calculation, use curves. As an example, if s is 0.5mg/L, U is ± 0.2 mg/L, and a 95% confidence level is desired, approximately 25 to 30 samples must be taken.

The above equation assumes that total error (population variability) is known. Total variability consists of the analytes of interest within the sampling site, collection, preservation, preparation, and analysis of samples, and data handling and reporting. In simpler terms, error (variability) can be divided into sampling and analysis components. Sampling error due to population variability (including heterogeneous distribution of analytes in the environment matrix) usually is much larger than analytical error components. Unfortunately, sampling error usually is not available and the analyst is left with only the published error of the measurement system (typically obtained by using a reagent water matrix under the best analytical conditions).

More accurate equations are available.[5] These are based on the Z distribution for determining the number of samples needed to estimate a mean concentration when variability is estimated in absolute terms using the standard deviation. The coefficient of variation (relative standard deviation) is used when variability is estimated in relative terms.

The number of random samples to be collected at a site can be influenced partly by the method that will be used. This values for standard deviation (SD) or relative standard deviation (RSD) may be obtained from each of the methods or in the literature.[6] However, calculations of estimated numbers of samples needed based only on this information will result in underestimated number of samples because only the analytical variances are considered, and the typically larger variances form the sampling operations are not included. Preferably, determine and use SDs or RSDs from overall sampling and analysis operations.

For estimates of numbers of samples needed for systematic sampling (e.g., drilling wells for sampling groundwater or for systematically sampling large water bodies such as lakes), equations are available that relate number of samples to shape of grid, area covered, and space between nodes of grid. The grid spacing is a complex calculation that depends on the size and shape of any contaminated spot (such as a groundwater plume) to be identified, in addition to the geometric shape of the sampling grid.

See individual methods for types and numbers of quality assurance (QA) and quality control (QC) samples, e.g., for normal-level (procedural) or low-level (contamination) bias or for precision, involving sampling or laboratory analysis (either overall or individually). Estimates of numbers of QC samples needed to achieve specified confidence levels also can be calculated. Rates of false positives (Type I error) and false negatives (Type II error) are useful parameters for estimating required numbers of QC samples. A false positive is incorrect conclusion that an analyte is present when it is absent & when it is present. If the frequency of false positives or false negatives desired to be detected is less than 10%, then

$$n = \ln \alpha / \ln (1-Y)$$

Where:
α = (1-desired confidence level), and
Y = frequency to detect (<10%).

If the frequency that is desirable to detect is more than 10%, iterative solution of a binomial equation is necessary.[5,8]

Equations are available as a computer program for computing sample number by the Z distribution, for estimating samples needed in systematic sampling, and for estimating required number of QC samples.

1.2.6 Sample Volumes

Collect a 1-L sample for most physical and chemical analysis. For certain determination, larger samples may be necessary. Table 1.2: I lists volumes ordinarily required for analyses, but it is strongly recommended that the laboratory that will conduct the analyses also be consulted to verify the analytical needs of sampling procedures as they pertain to the goals and data quality objective of an investigation.

Do not use samples from the same container for multiple testing requirements (e.g., organic, inorganic, radiological, bacteriological, and microscopic examinations) because methods of collecting and handling are different for each type of test. Always collect enough sample volume in the appropriate container in order to comply with sample handling, storage, and preservation requirements.

1.2.7 References

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TABLE 1.2: I SUMMARY OF SPECIAL SAMPLING AND HANDLING REQUIREMENTS

Determination	Container†	Minimum Sample Size mL	Sample Type‡	Preservation	Maximum Storage	
					Recommended	Regulatory‖
Acidity	P,G(B)	100	g	Refrigerate	24h	14d
Alkalinity	P,G	200	g	Refrigerate	24h	14d
BOD	P,G	1000	g,c	Refrigerate	6h	48h
Boron	P (PTEF) or quartz	1000	g,c	HNO_3 to pH<2	28d	6 months
Bromide	P,G	100	g,c	None required	28d	28d
Carbon organic total	G (B)	100	g,c	Analyze immediately; or refrigerate and add HCL, H_3PO_4, or H_2SO_4 to pH<2	7d	28d
Carbon dioxide	P,G	100	g	Analyze immediately	0.25h	N.S.
COD	P,G	100	g,c	Analyze as soon as possible, or add H_2SO_4 to pH<2; refrigerate	7d	28d
Chloride	P,G	50	g,c	None required	N.S.	28d
Chlorine, total, residual	P,G	500	g	Analyze immediately	0.25h	0.25h

Chlorine dioxide	P,G	500	g	Analyze immediately	0.25h	N.S.
Chlorophyll	P,G	500	g	Unfiltered, dark, 4°C	24-48h	
				Filtered, dark, -2°C (Do not store in frost-free freezer)	28d	
Color	P,G	500	g,c	Refrigerate	48h	48h
Specific conductance	P,G	500	g,c	Refrigerate	28d	28d
Cyanide Total	P,G	1000	g,c	Add NaOH to pH>12, refrigerate in dark#	24h	14d;24h if sulfide present
Amenable to chlorination	P,G	1000	g,c	Add 0.6g ascorbic acid if chlorine is present and refrigerate	Stat	14d; 24h if sulfide present
Fluoride	P	100	g,c	None required	28d	28d
Hardness	P,G	100	g,c	Add HNO_3 or H_2SO_4 to pH<2	6 months	6 months
Iodine	P,G	500	g	Analyze immediately	0.25h	N.S.
Metals, general	P(A), G(A)	1000	g,c	For dissolved metals filter immediately, add HNO_3 to pH <2	6 months	6 months
Chromium VI	P(A), G(A)	1000	g	Refrigerate	24h	24h
Copper by colorimetry*			g,c			
Mercury	P(A), G(A)	1000	g,c	Add HNO_3 to pH<2, 4°C, refrigerate	28d	28d

Nitrogen Ammonia	P,G	500	g,c	Analyze as soon as possible or add H_2SO_4 to pH<2, refrigerate	7d	28d
Nitrate	P,G	100	g,c	Analyze as soon as possible; refrigerate	48h	48h (28d for chlorinated samples)
Nitrate + nitrite	P,G	200	g,c	Add H_2SO_4 to pH<2, refrigerate	1-2d	28d
Nitrite	P,G	100	g,c	Analyze as soon as possible; refrigerate	none	48h
Organic, Kjeldahl*	P,G	500	g,c	Refrigerate, add H_2SO_4 to pH<2	7d	28d
Odor	G	500	g	Analyze as soon as possible; refrigerate	6h	N.S.
Oil and grease	G, wide-mouth calibrated	1000	g	Add HCl or H_2SO_4 to pH<2, refrigerate	28d	28d
Organic compounds MBAs	P,G	250	g,c	Refrigerate	48h	N.S.
Pesticides*	G(S), PTEF-lined cap	1000	g,c	Refrigerate, add 1000mg ascorbic acid/L if residual chlorine present	7d	7d until extraction; 40 d after extraction
Phenols	P,G, PTEF-lined cap	500	g,c	Refrigerate, add H_2SO_4 to pH<2	*	28d until extraction

Purgeables* by purge and trap	G, PTEF-lined cap	2 X 40	g	Refrigerate; add HCl to pH<2; add 1000 mg ascorbic acid/L if residual chlorine present	7d	14d
Base/neutrals & acids	G(S) amber	1000	g,c	Refrigerate	7d	7d until extraction; 40d after extraction
Oxygen, dissolved Electrode Winkler	G, BOD bottle	300	g	Analyze immediately Titration may be delayed after acidification	0.25h 8h	0.25h 8h
Ozone	G	1000	g	Analyze immediately	0.25h	N.S.
pH	P,G	50	g	Analyze immediately	0.25h	0.25h
Phosphate	G(A)	100	g	For dissolved phosphate filter immediately; refrigerate	48h	N.S
Phosphorus, total	P,G	100	g,c	Add H_2SO_4 to pH<2 and refrigerate	28d	
Salinity	G, wax seal	240	g	Analyze immediately or use wax seal	6 months	N.S
Silica	P (PTEF) or quartz	200	g,c	Refrigerate, do not freeze	28d	28d
Sludge digester gas	G, gas bottle		g		N.S.	
Solids[9]	P,G	200	g,c	Refrigerate	7d	2-7d; see cited reference
Sulfate	P,G	100	g,c	Refrigerate	28d	28d

Sulfide	P,G	100	g,c	Refrigerate; add 4 drops 2N zinc acetate/100 mL; add NaOH to pH>9	0.25h	0.25h
Temperature	P,G		g	Analyze immediately	0.25h	0.25h
Turbidity	P,G	100	g,c	Analyze same day; store in dark up to 24h, refrigerate	24h	48h

* For determination 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 backed.

‡ g = grab; c = composite

§ Refrigerate = storage at 4°C ± 2°C; in the dark; analyze immediately = analyze usually within 15 min of sample collection.

‖ See citation[10] for possible differences regarding container and preservation requirements. N.S. = not stated in cited reference; stat = no storage allowed; analyze immediately.

If sample is chlorinated, see text for pretreatment.

1.3 Sample Storage and Preservation

Complete and unequivocal preservation of samples, whether domestic wastewater, industrial wastes, or natural waters, is a practical impossibility because complete stability for every constituent never can be achieved. At best, preservation techniques only retard chemical and biological changes that inevitably continue after sample collection.

1.3.1 Sample Storage before Analysis

a. *Nature of sample changes:* Some determinations are more affected by sample storage than others. Certain cations are subject to loss by adsorption on, or ion exchange with, the walls of glass containers. These include aluminium, cadmium, chromium, copper, iron, lead, manganese, silver and zinc, which are best collected in a separate clean bottle and acidified with nitric acid to a pH below 2.0 to minimize

precipitation and adsorption on container walls. Also, some organics may be subject to loss by adsorption to the walls of glass containers.

Temperature changes quickly; pH may change significantly in a matter of minutes; dissolved gases (oxygen, carbon dioxide) may be lost. Because in such basic conductance, turbidity, and alkalinity immediately after sample collection. Many organic compounds are sensitive to changes in pH and/or temperature resulting in reduced concentrations during storage.

Changes in the pH-alkalinity-carbon dioxide balance may cause calcium carbonate to precipitate, decreasing the values for calcium and total hardness.

Iron and manganese are readily soluble in their lower oxidation states but relatively insoluble in their higher oxidation states; therefore, these cations may precipitate or they may dissolve from sediment, depending on the redox potential of the sample. Microbiological activity may affect the nitrate-nitrite-ammonia content, phenol or BOD concentration, or the reduction of sulfate. Residual chlorine is reduced to chloride. Sulfide, sulfite, ferrous iron, iodide, and cyanide may be lost through oxidation. Color, odor, and turbidity may increase, decrease, or change in quality. Sodium, silica, and boron may be leached from the glass container. Hexavalent chromium may be reduced to trivalent chromium.

Biological activity taking place in a sample may change the oxidation state of some constituents. Soluble constituents may be converted to organically bound materials in cell structures, or cell, lysis may result in release of cellular material into solution. The well-known nitrogen and phosphorus cycles are examples of biological influences on sample composition.

Zero head-space is important in preservation of samples with volatile organic compounds and radon. Avoid loss of volatile materials by collecting sample in a completely filled container. Achieve this by carefully filling the bottle sot that top of meniscus is above the top of the bottle rim. It is important to avoid spillage or air entrapment if preservatives such as HCl or ascorbic acid have already been added to the bottle. After capping or sealing bottle, check for air bubbles by inverting and gently tapping it; if one or more air bubbles are observed then, if practical, discard the same and repeat refilling bottle with new sample until no air bubbles are observed (this cannot be done if bottle contained preservatives before it was filled).

Serum vials with septum caps are particularly useful in that a sample portion for analysis can be taken through the cap by using a syringe,[1] although the effect of pressure reduction in the head-space must be considered. Pulling a sample into a syringe under vacuum can result in low bias data for volatile compounds and the resulting headspace precludes taking further subsamples.

b. *Time interval between and analysis:* In general, the shorter the time that elapses between collection of a sample and its analysis, the more reliable will be the

analytical results. For certain constituents and physical values, immediate analysis in the field is required. For composited samples it is common practice to use the time at the end of composite collection as the sample collection time.

Check with the analyzing laboratory to determine how much elapsed time may be allowed between sample collection and analysis; this depends on the character of the sample and the stability of the target analytes under the conditions of storage. Many regulatory methods limit the elapsed time between sample collection and analysis (see table 1.2: I). Changes caused by growth of microorganisms are greatly retarded by keeping the sample at a low temperature (<4°C but above freezing). When the interval between sample collection and analysis is long enough to produce changes in either the concentration or the physical state of the constituent to be measured, follow the preservation practices given in Table 1.2: I. Record time elapsed between sampling and analysis, and which preservative, if any, was added.

1.3.2. Preservation Techniques

To minimize the potential for volatilization or biodegradation between sampling and analysis, keep samples as cool as possible without freezing. Preferably pack samples in crushed or cubed ice or commercial ice substitutes before shipment. Avoid using dry ice because it will freeze samples and may cause glass containers to break. Dry ice also may effect a pH change in samples. Keep composite samples cool with ice or a refrigeration system set at 4°C during compositing. Analyze samples as quickly as possible on arrival at the laboratory. If immediate analysis is not possible, preferably store at 4°C.[1]

No single method of preservation is entirely satisfactory; choose the preservative with due regard to the determinations to be made. Use chemical preservatives only when they do not interfere with the analysis being made. When they are used, add them to the sample bottle initially so that all sample portions are preserved as soon as collected. Because a preservation method for one determination may interfere with another one, samples for multiple determinations may need to be split and preserved separately. All methods of preservation may be inadequate when applied to suspended matter. Do not use formaldehyde as a preservative for samples collected for chemical analysis because it affects many of the target analytes.

Methods of preservation are relatively limited and are intended generally to retard biological action, retard hydrolysis of constituents.

Preservation methods are limited to pH control, chemical addition, the use of amber and opaque bottles, refrigeration, filtration, and freezing.

The foregoing discussion is by no means exhaustive and comprehensive. Clearly it is impossible to prescribe absolute rules for preventing all possible changes. Additional advice will be found in the discussions under individual determinations, but to a larger degree the dependability of an analytical determination rests on the experience and good judgment of

the person collecting the sample. Numbers of samples required for confidence levels in data quality objectives, however, rely on statistical equations such as those discussed earlier.

1.3.3. Bibliography

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2

METHODS OF REAGENT WATER

2.1 REAGENT WATER

2.1.1 Introduction

One of the most important aspects of analysis is the preparation of reagent water to be used for dilution of reagents and for blank analysis. Reagent water is water with no detectable concentration of the compound or element to be analyzed at the detection level of the analytical method. Reagent water should be free of substances that interfere with analytical methods. The quality of water required is related directly to the analysis being made. Requirements for water quality may differ for organic, inorganic, and biological constituents depending on the use(s) for which the water is intended.
Any method of preparation of reagent water is acceptable provided that the requisite quality can be met. Improperly maintained systems may add contaminants. Reverse osmosis, distillation, and deionization in various combinations all can produce reagent water when used in the proper arrangement. Ultrafiltration and/or ultraviolet treatment also may be used as part of the process. Table 2.1: I lists commonly available processes for water purification and major classes of contaminants removed by purification.

For details on preparing water for microbiological tests:-

Commercial systems are available that include some combination of prefiltration, activated carbon, mixed-bed resins, and reverse-osmosis with final filtration to produce a reagent-grade water. The life of such systems can be extended greatly if the source water is pretreated by distillation or by reverse osmosis to remove dissolved solids. Such systems tend to produce the same quality water until resins or activated carbon are near exhaustion and quality abruptly becomes unacceptable3. Some deionization components are available now that automatically regenerate the ion exchange resins. Do not store reagent water unless a commercial UV irradiation device is installed and is confirmed to maintain sterility. Monitor reagent water continuously or daily with a calibrated conductivity meter and analyze at least annually for trace metals. Replace cartridges at intervals recommended by the manufacturer based on the estimated usage and source water quality. Do not wait for column failure. If bacteria-free water is desired, include aseptic final filtration with a 0.22-µm-pore membrane filter and collect in a sterile container. Monitor treated water for contamination and replace the filter as necessary.

TABLE 2.1: I. WATER PURIFICATIO PROCESSES

Process	Major Classes of Contaminants*					
	Dissolved Ionized Solids	Dissolved Ionized Gases	Dissolved Organics	Particulates	Bacteria	Phrogens/ Endotoxins
Distillation	G-E	P	G	E	E	E
Deionization	E	E	P	P	P	P
Reverse osmis	G	P	G	E	E	E
Carbon adsorption	P	P§	G-E‖	P	P	P
Filtration	P	P	P	E	E	P
Ultrafiltration	P	P	G#	E	E	E
Ultraviolet oxidation	P	P	G-E**	P	G‡‡	P

Permission to use this table from C3-A2, Vol. 11, No. 13, Aug. 1991, "Preparation and Testing of Reagent Water in the Clinical Laboratory- Second Edition" has been granted by the National Committee for Clinical Laboratory Standards. The complete current standard may be obtained from National Committee for Clinical Laboratory Standards, 771 E. Lancaster Ave., Villanova, PA 19058.

* E = Excellent (capable of complete or near total removal), G = Good (capable of removing large percentages), P = Poor (little or no removal).

Resistivity of water purified by distillation is an order of magnitude less than water produced by deionization, due mainly to the presence of CO_2 and sometimes H_2S, NH_3, and other ionized gases if present in the feedwater resistivity.

Resistivity of dissolved ionized solids in the product water depends on original feedwater resistivity.

§ Activated carbon removes chlorine by adsorption.

‖ When used in combination with purification processes, special grades of activated carbon and other synthetic adsorbents exhibit excellent capabilities for removing organic contaminants. Their use, however, is targeted toward specific compounds and applications.

Ultrafilters have demonstrated usefulness in reducing specific feedwater organic contaminants based on the rated molecular weight cut-off of the membrane.

**185 nm ultraviolet oxidation (batch process systems) is effective in removing trace organic contaminants when used as post-treatment. Feedwater makeup plays a critical role in the performance of these batch processors.

‡‡254 nm UV sterilizers, while not physically removing bacteria, may have bactericidal or bacteriostatic capabilities limited by intensity, contact time, and flow rate.

2.2 METHODS OF PREPARATION OF REAGENT WATER

2.2.1. Distillation

Prepare laboratory-grade distilled water by distilling water from a still all-borosilicate glass, fused quartz, tin, or titanium. To remove ammonia distill from an acid solution. Remove CO_2 by boiling the water for 15 min and cooling rapidly temperature; exclude atmospheric CO_2 by using a tube containing soda lime or a commercially available CO_2-removing agent.

Boiling the water may add other impurities by leaching impurities from the container. Freshly replaced filters, cartridges and resins initially can release impurities. Pretreat feedwater and provide periodic maintenance to minimize scale formation within the still. Pretreatment may be required where the feed-water contains significant concentrations of calcium, magnesium, and bicarbonate ions; it may involve demineralization via reverse osmosis or ion exchange.

2.2.2. Reverse Osmosis

Reverse osmosis is a process in which water is forced under pressure through a semipermeable membrane removing a portion of dissolved constituents and suspended impurities. Product water quality depends on feedwater quality.

Select the reverse osmosis membrane module appropriate to the characteristics of the feedwater. Obtain rejection data for contaminants in the operating pressure to be used in preparing reagent water. Set overall water production to make the most economical use of water without compromising the final quality of the permeate. Selection of spiral-wound or hollow fiber configurations depends on fouling potential of the feedwater. Regardless of configuration used, pretreatment may be required to minimize membrane fouling with colloids or particulates and to minimize introduction of chlorine, iron, and other oxidizing compounds that may degrade reverse osmosis membranes. Periodic flushing of the membrane modules is necessary.

2.2.3. Ion Exchange

Prepare deionized water by passing feedwater through a mixed-bed ion exchanger, consisting of strong anion and strong cation resins mixed together. When the system does not run continuously, recirculate product water under ion-exchange bed.

Use separate anion cation resin beds in applications where resin regeneration is economically attractive. In such instances, position the anion exchanger downstream of the cation exchanger to remove leachates from the cation resin. Proper bed sizing is critical to the performance of the resins. In particular, set the length-to-diameter ratio of the bed in accordance with the maximum process flow rate to ensure that optimal face velocities are not exceeded and that sufficient residence time is provided.

In applications where the feedwater has significant quantities of organic matter, remove organics to minimize potential fouling of the resins. Possible pretreatments include prefiltration, distillation, reverse osmosis, or adsorption.

2.2.4. Adsorption

Adsorption is generally used to remove chlorine and organic impurities. It is accomplished typically with granular activated carbon. Efficiency of organics removal depends on the nature of the organic contaminants, the physical characteristics of the activated carbon, and the operating conditions. In general, organics adsorption efficiency in inversely proportional to solubility and may be inadequate for the removal of low-molecular-weight, polar compounds. Performance differences among activated carbons are attributable to the use of different raw materials and activation procedures. Select the appropriate activated carbon with regard to these differences. Even with optimum activated carbon, proper performance will not be attained unless the column is sized to give required face velocity and residence time at the maximum process flow rate.

Use of activated carbon may adversely affect resistivity. This effect may be controlled by use of the lowest level of organic contamination, use mixtures of polishing resins with special carbons in conjunction with additional treatment steps, such as reverse osmosis, natural carbons, ultraviolet oxidation, or Ultrafiltration.

2.3 REAGENT WATER QUALITY

2.3.1. Quality Guidelines

Several guidelines for reagent water quality, based on contaminant levels, are available, but the final test is the appropriateness for the analysis. Table 2.3: I lists some characteristics of various qualities of reagent water.

High-quality reagent water, having a minimum resistivity of 10 megohms-cm, 25°C (in line), typically is prepared by distillation, deionization, or reverse osmosis treatment of feedwater followed by polishing with a mixed-bed deionizer and passage through a 0.2µm-pore membrane filter. Alternatively treat by reverse osmosis followed by carbon adsorption and deionization. Determine quality at the time of production. Mixed-bed deionizers typically add small amounts of organic matter to water, especially if the beds are fresh. Resistivity should be >10 megohm-cm at 25°C, measured in-line. Resistivity measurements will not detect organics or nonionized contaminants, nor will they provide an accurate assessment of ionic contaminants at the microgram-per-liter level.

Medium-quality water typically is produced by distillation or deionization. Resistivity should be >1 megohm-cm at 25°C.

TABLE 2.3: I REAGENT WATER SPECIFICATIONS*

Quality Parameter	High	Medium	Low
Resistivity, megohm-cm at 25°C	>10	>1	0.1
Conductivity, µmho/cm at 25° C	<0.1	<1	10
Sio_2, mg/L	<0.05	<0.1	<1

Low-quality water should have a minimum resistivity of 0.1 megohm-cm, and may be used for glassware washing, preliminary rinsing of glassware, and as feedwater for production of higher-grade waters.

The pH of high-or medium-quality water cannot be measured accurately without contaminating the water. Measure other constituents as required for individual tests.

High-quality water cannot by stored without significant degradation; produce it continuously and use it immediately after processing. Medium-quality water may be stored, but keep storage to a minimum and provide quality consistent with the intended use. Store only in materials that protect the water from contaminations, such as TFE and glass for organics analysis or plastics for metals. Store low-quality water in materials that protect the water from contamination.

2.3.2. Bibliography

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3

MICROBIOLOGICAL ANALYSIS

3.1 PREAMBLE

Water borne pathogens can enter the human body through intact or compromised skin, inhalation, ingestion, aspiration, or by direct contact with mucosa of the eye, ear, nose, mouth and genitals, and cause disease. Besides failure of potable water systems, which usually contribute to the greatest number of outbreaks of waterborne diseases, common outdoor recreational activities such as swimming, boating, bathing, camping and hiking, all place humans at risk of waterborne diseases from ingestion or direct contact with contaminated water. A risk analysis approach is required for overcoming the problems with waterborne diseases and water quality monitoring. While this could be achieved relatively easily with respect to chemical contaminants, and standards could be set up for chemical contaminants, setting standards for microbial contaminants has proved more difficult, because, (a) methods for detection of many pathogens are not yet available; (b) days to weeks are some times required to obtain results; and (c) expensive and time consuming methods are often required to detect and quantify the presence of specific pathogens.

To overcome these difficulties, the concept of testing water samples for indicator organisms was introduced as far back as the turn of the 19th century. The concept was developed for assessing faecal contamination of water and the concept relies on the fact that certain non-pathogenic bacteria present in water can be easily isolated and quantified by simple bacteriological methods; detection of these bacteria would essentially mean that faecal contamination has occurred and also suggest that enteric pathogens also may be present. The essential criteria for indicator organisms are given in the box below:

Criteria for indicator micro-organisms in water

- Should be useful for all types of water
- Should be present whenever enteric pathogens are present
- Should have a reasonable longer survival time than the hardest enteric pathogen
- Should not grow in water
- Testing method for the organism should be easy to perform
- Density of the indicator should have some direct relationship to the degree of faecal pollution
- Should be a member of the intestinal micro-flora of humans and warm-blooded animals

It should, however, be appreciated that the choice of monitoring indicator(s) presupposes an understanding of the parameters to be measured and the relationship of the indicator(s) to the pathogen(s). No single indicator provides assurance that water is pathogen-free. Pathogens such as *Psedomonas, Aeromonas, Plesidomnas, Yersinia, Vibrio, Legionella and Mycobacterium* may not correlate with the most commonly used coliform indicators, and also, traditional bacteria indicators may not also correlate with viruses and parasites in pristine waters or ground waters. A brief consideration of the common indicator organisms that are currently employed in qualitative/quantitative tests for water would be appropriate here.

Indicator microòrganisms of faecal pollution:

3.1.1. Coliform bacteria:

Coliform bacteria include all aerobic and facultative anaerobic gram negative, non-sore forming, rod-shaped bacteria that produce gas upon fermentation in prescribed culture media within 48 hr at 35°C.

In recent years, new criteria have been added to traditional definition of coliform bacteria and E. *coli*, using the presence of characteristic enzymatic activities. Definition of coliform or faecal coliform basically relies on the activity of a single enzyme β- *galactosidase.* The new enzymatic definition of TOTAL COLIFORM bacteria is based on the presence of β-*galactosidase;* and that of E. *coli* is based on the enzymatic action of β- *glucuronidase.* This aspect is well utilized in developing very sensitive and specific presence-absence (P-A) tests for detection of total coliforms and E *coli.*

Deficiencies with the use of coliform bacteria as indicator of water quality include:

- Regrowth in aquatic environments
- Regrowth in distribution systems, including biofilm colonization
- Suppression by high background bacterial growth
- Not indicative of health threat
- No relationship with enteric protozoan and viral concentration.

Coliform bacteria had been used historically to assess the microbial quality of drinking water. However, by the 1980s it had become quite clear that coliform bacteria did not indicate the presence of pathogenic waterborne *Giardia* or enteric viruses. Numerous outbreaks had occurred. In which coliform standards were met, because of greater resistance of viruses and *Giardia* to chlorination. A new approach was needed to ensure the microbial safety of drinking water.

Most Probable Number (MPN), the Membrane filter (MF) and presence-absence (P/A) tests are used to detect the total coliforms.

3.1.2. Faecal coliform bacteria:

Faecal coliform bacteria are differentiated in the laboratory by their ability to ferment lactose, with production of acid and gas at 44.5°C within 24 h.

Faecal coliforms pose some of the same limitations as those posed by coliforms (Regrowth in distribution system, less resistance to water treatment than viruses and protozoa, etc.)

Faecal coliforms are also detected by similar methods (MPN, MF and P/A) used for total coliforms.

3.1.3. Faecal streptococci:

Faecal streptococci include the gram-positive, Lancefield Group-D streptococci, belonging to the genera *Enterococcus* and *Streptococcus*.

The genus *Enterococcus* is differentiated from other streptococci by ability to grow in 6.5% NaCl, pH 9.6 and 45°C (includes spp. E. *avivum*, E. *faecium*, E. *faecalis* and E. *gallinarium*). E. faecium and E. faecalis are more specific to human gut.

In the genus *Streptococcus*, only S. *bovis* and S. *equines* are considered to be true faecal streptococci have certain advantages over the coliforms, as indicators:

- They rarely multiply in water
- More resistant to environmental stress and chlorinated that coliforms
- Persist longer in the Environment
- Suggested as useful indicators for recreational waters

Both MPN and MF methods may be used for isolation and enumeration of faecal streptococci.

3.1.4. Clostridium perfringens:

They are sulphite-reducing anaerobic spore-formers, gram-positive, rod-shaped and exclusively of faecal origin. The hardly spores (resistant to 75°C for 15 min.) limit its usefulness as an indicator; it could be used as an indicator of past pollution and an indicator of removal of protozoan parasites and viruses during treatment of drinking water and wastewater.

3.1.5. Pseudomonas aeruginosa:

They are gram-negative, non-sporulating, rod-shaped bacteria, producing characteristics pyocyanin (blue-green) and the green fluorescent pigment fluorescein. One of the most common opportunistic pathogens. Numerous cases of folliculitis, dermatitis, ear and urinary

tract infections are due to P. *aeruginosa,* associated with swimming in contaminated waters. Because of this association and also its consistent presence in high numbers in sewage, this organism has been suggested as an indicator for water quality in swimming pools, hot tubs and other recreational waters. But its value as an indicator is very little, because it is ubiquitous in nature and can multiply under natural conditions (can grow even in distilled water).

3.1.6. Hydrogen sulphide (H_2S)-producing bacteria:

H_2S-producing organisms (bacteria) are invariably present in faeces. There is a very strong correlation between presence of H_2S-producing bacteria and faecal pollution of water. By detecting the presence of H_2S-producing bacteria in water samples, faecal pollution of such waters can be deduced.

3.1.7. Coliphages and other bacteriophages:

Their use as indicators of faecal pollution is based on the assumption that their presence in water samples denotes the presence of bacteria capable of supporting replication of the phages. Two groups of phages have been studied for their suitability as indicator organisms:

- Somatic Coliphages that infect E. *coli* host strains through cell wall receptors.
- F-specific RNA coliphage-which infect strains of E. *coli* and related bacteria through the F + or sex pili

Advantages of using coliphage is that they can be detected by simple and inexpensive techniques, in 8-18 hr. Both plating (agar-overlay method) and MPN method can be used to detect coliphages, in volumes ranging from 1-100 mL of water.

Another bacteriophages of interest is the bacteriophages that infect *Bacteriodes fragilis.* These bacteria (Bacteriodes spp.) are strict anaerobes and are a major component of the human faecal microflora. The bacteriophages that infect *Bacteriodes fragilis* appear to be exclusively of human origin and can serve as an ideal indicator organism. The main disadvantage, however, lies with the fact that since the host organism (B. *fragilis*) is an anaerobic organism, the detection of the indicator organism would involve tedious and complicated methodologies.
The coliphages and other bacteriophages have also been suggested as indicators of viral pollution. This is because the structure, morphology and size, as well as their behaviour in the aquatic environment closely resemble those of enteric viruses. These bacteriophages are quite useful in evaluation of virus resistance to disinfectants.

An essential goal for the provision of safe drinking water is that it be essentially free of (at low risk of containing) disease-causing micro-organisms. Since the beginning of the 20th century, the detection of faecal indicator bacteria in drinking water has been used as the bases of criteria, guidelines and standards for acceptable limits of faecal contamination and as the basis for judging or predicting the possible presence or absence or pathogenic (disease-

causing) micro-organisms. The WHO Guidelines for Drinking Water Quality (GDWQ) and many other authorities continue to support the use of bacterial indicator levels and their measurement as a basis for judging and verifying drinking water quality. However, such faecal indicator analysis of drinking water as a measure of end-product quality and determinant of microbial disease risk is only one of many measures and activities in an overall system for providing safe drinking water.

It is now well established that waterborne pathogens enter the drinking water systems through faecal contamination, and the list of such organisms (which include bacteria, viruses, protozoans and parasites) is a very long one. It would neither be practicable, nor is it necessary, to test a sample of drinking water for all the pathogens that might possible be present in it, as that would involve many different procedures, a multitude of media and other materials, incubation conditions, etc. It would, therefore, be sufficient if we can determine whether the water sample was contaminated with faces, such water is not fit for human consumption, as there would be a possibility that such water might harbour pathogenic organisms. Therefore, by establishing faecal contamination of water, we will be able to infer upon its potabilility or otherwise.

In the laboratory there are three simple procedures of testing water samples for faecal contamination:

1. H_2S-Strip method: This is a simple, reliable and easy-to-perform (by even untrained personnel), 'Presence / Absence' test for bacteriological quality, which works on the principle that there is a close correlation between faecal contamination and the presence of hydrogen sulphide (H_2S)-producing bacteria and, the faecal pollution of water can be deduced by demonstration of H_2S production. It has been claimed, by various workers, that the H_2S-strip method shows >90% agreement with the conventional MPN test.

2. Most Probable Number (MPN) method for coliform bacteria-using the multiple tube fermentation technique: In this method the MPN of total coliform bacteria, faecal coliform bacteria, faecal coliform bacteria (or the thermotolerant coliforms) present in the water sample is determined, along with the presence / absence of *Escherichia coli.*

3.2 COMPOSITION OF COMMON BACTERIOLOGICAL CULTURE MEDIA

3.2.1. MacConkey broth

Double-strength medium:

- Dissolve the peptone, sodium chloride and bile salts in the water by heating and store at 4°C overnight.
- Filter while still cold, add the lactose and dissolve.

- Adjust to pH 7.4 ± 0.2 and add the Neutral Red.

Single-strength medium:

- Prepare single-strength medium by dilution of the double-strength medium with an equal volume of distilled water or make separatory using half the concentration of ingredients.
- Distribute single-strength medium in 5 mL volumes and double-strength medium in 10 mL and 50 mL volumes. Each tube or bottle used should contain an inverted fermentation (Durham) tube.
- Autoclave at 115°C for 10 min.

3.2.2. Brilliant Green Lactose Bile Broth:

- Dissolve peptone and lactose in 500 mL distilled water
- Add 20 g dehydrated oxgall dissolved in 200 mL distilled water. The pH of this solution should be 7.0-7.5
- Mix and add water to make 975 brilliant green in distilled water
- Add distilled water to make 1 litre
- Dispense into fermentation tubes, making certain that fluid level covers inverted vials. Autoclave 15 min at 121°C. Final pH, 7.2 ± 0.1.

3.2.3. EC Broth:

- Distribute 8 mL portions to 16 x 150 mm test tubes containing inverted fermentation (Durham) tubes
- Autoclave 15 min at 121°C. Final pH, 6.9 ± 0.2.

3.2.4. EC-MUG medium:

- Prepare as for EC Broth above, but add 50 mg 4-methylumbelliferyl-β-D-glucuronide (MUG) per litre before autoclaving (15 min, 121°C)
- Do not use Durham tubes, as gas is not relevant to the test and could interfere with the interpretation of the UV light results.

3.2.5. Indole medium:

- Adjust pH to 7.3 ± 0.2.
- Dispense 4 mL portions to
- Autoclave 15 min at
- Final pH, 7.2 ± 0.2.

3.2.6. MacConkey agar: (w/o crystal vilet and NaCl, w/Sodium taurocholate)

- Suspended ingredients and hear with agitation to dissolve
- Boil 1-2 min
- Autoclave 15 min at 121°C
- Cool to 45-50°C, pour 20 mL portions into sterile 15 x 100 mm Petri dishes
- Dry at room temperature with lids closed
- Final pH, 7.1 ± 0.2.

3.3 TEST PROCEDURES

A. Multiple tube fermentation technique for coliform bacteria (MPN test):

In the multiple-tube method, a series of tubes containing a suitable selective broth culture medium (lactose-containing broth, such as MacConkey broth) is inoculated with test portions of a water sample. After a specified incubation time at a given temperature, each tube showing gas formation is regarded as "presumptive positive" since the gas indicates the possible presence of coliforms. However, gas may also be produced by other organisms, and so a subsequent confirmatory test is essential. The two tests are known respectively as the presumptive test and the confirmatory test.

For the confirmatory test, a more selective culture medium (brilliant green bile broth) is inoculated with material taken from the positive tubes. After an appropriate incubation time, the tubes are examined for gas formation as before. The most probable number (MPN) of bacteria present can then be estimated from the number of tubes inoculated and the number of positive tubes obtained in the confirmatory test. Using specially devised statistical tables. This technique is known as the MPN method

Equipment and supplies:

- Culture tubes containing inverted vials (Durham tubes): each tube should be large enough for a vial, completely filled with medium, to be submerged in it.
- Gas burner: a Bunsen or similar burner is adequate
- Inoculation loop and holder: lengths of 2 or 26 gauge wire (7.5-10 cm) should be used. Nichrome wire is acceptable, but platinum-iridium is better. The wire is set in a handle made of metal or glass, of diameter similar to that of a pencil. To make the inoculation loop, the wire is bent to form a circle 3-4 mm in diameter.
- Clearing and maintenance equipment: items such as brushes for cleaning tubes, bottles, etc., a waste bin, and a tool kit are required.
- Safety equipment: there should be an adequate first-aid and a fire extinguisher or other means of fire control in every laboratory.

The following consumable items are required:

- Culture media / Reagents: the following culture media and reagents are required
  - MacConkey Broth with neutral red (double strength)
  - MacConkey Broth with neutral red (single strength)
  - Brilliant Green Blue broth (BGB)
  - Tryptone water/Peptone water (for indole test)
  - Kovac's Reagent
- Laboratory disinfectant: for cleaning laboratory surfaces and the pipette discard bin.
- Detergent: for washing glassware, etc
- Sodium thiosulphate solution: required when chlorinated supplies are tested. Sodium thiosulphate neutralizes any residual chlorine in samples at the time of collection, preventing it from acting on any micro-organisms present in water samples.
- Autoclave tape

Culture media preparation

- Commercially available dehydrated media simplify the preparation of culture broths and are therefore recommended for laboratory work. Various manufacturers produce these media as powders, which can then be easily weighed, dissolved in distilled water, and dispensed into culture tubes before sterilization.

Preparation of media: Media should be prepared in accordance with the manufacturer's instruction, as follows:

a. Dissolve the stated amount of the dehydrated medium in distilled water to obtain the double-strength or single-strength presumptive medium (MacConkey broth). The confirmatory medium (BGB) is required only in single-strength.

b. Dispense the requisite volume into culture tubes containing an inverted Durham tube, and cap the culture tubes; simple cotton plugs may be used or more ideally metal slip-on caps may be used to cap the tubes.

c. Sterilise in an autoclave at 115°C for 10 minutes (or in accordance with the manufacturer's specifications). It is particularly important that media containing disaccharides, e.g. lactose, are not autoclave at higher temperatures.

d. The sterilized medium may be stored at room temperature (approximately 25°C) or, ideally, at 2-8°C. Media should in any case be warmed to room temperature before use to ensure that all components have re-dissolved. In addition, since several dyes are light sensitive, the solution should be protected form exposure to light.

Procedure for inoculation of samples: The procedure to be used for testing relatively unpolluted water, such as treated water from waterworks or distribution system or end user's tap.

Note down the details of the sample collected (from the label on the bottle) in the register.

a. With the stopper in position, shake the bottle vigorously to achieve a homogeneous dispersion of bacteria. (If the bottle is completely full, remove the stopper and discard about 20-30 mL of water; then replace the stopper and shake. This ensures thorough mixing).

b. Add 50 mL of sample to a tube / flask containing 50 mL of presumptive broth (double strength). With a sterile 10 mL pipette, inoculate 10 mL of the sample into each the five tubes containing 5 mL presumptive broth (single strength). It is advisable to shake the tubes gently to distribute the sample uniformly throughout the medium. Be careful as to not shake so hard that air is introduced into the inverted tubes.

c. Incubate the tubes at 35° C ± 5°C for 24 hours.

d. At the end of the 24-hour incubation period, examine each tube for the presence of gas. If present, gas can be seen in the Durham tube. If none is visible, gently shake the tube; if any effervescence (streams of tine bubbles) is observed, the tubes should be considered positive.

e. Record the number of positive tubes after 24 hours.

f. Re-incubate negative tubes for a further 24-hour period. At the end of this period, check the tubes again for gas production as in 5 above. Gas production at the end of either 24 or 48 hours' incuba-tion is presumed to be due to the presence of coliforms in the sample.

g. Record the number of positive tubes after 48 hours.

h. The confirmatory test should be carried out at the end of both the 24-hour and the 48-hour incubation. Using a sterile loop, transfer one or two loops-full from each presumptive positive tube into tow tubes containing respectively confirmatory broth (BGB) and tryptone water. (Sterilise the inoculation loop before each transfer by flaming and allow cooling). To confirm the presence of thermotolerant coliforms, incubate the subculture tubes from each presumptive positive tube for 24 hours at 44.5 ± 0.5°C.

   [Alternatively, transfer a loopful of a positive MacConkey broth tube into BGB medium and incubate at 35°C for 24-48 hours. This will be a better confirmation of Total Coliforms.]

i. At the end of 24 hours' incubation, examine each broth tube for growth and the presence of gas in the Durham tube. Record the results, as done previously.

j. To each tube of tryptone water, add approximately 0.1 mL of Kovacs reagent and mix gently. The presence of indole is indicated by a red colour in the Kovacs reagent, forming a film over the aqueous phase of the medium.

k. Confirmatory tests positive for indole, growth, and gas production show the presence of E. coli. Growth and gas production in the presence of indole confirms thermotolerant coliforms.

Determination of MPN: For treated water, where one 50 mL, five 10 mL and five 1 mL portions are inoculated, the MPN can be found from the test results by referring to the MPN Table (also called McCrady's Table) given in Annexure-G. It would be best to use the numbers from the confirmatory tests for the determination of water quality (Table 3.3: I).

Table 3.3: I: Water Samples are classified based on the presumptive count in the following way:

Class	Grading	Coliform Count / 100 mL (MPN)	E. *coli* cont per 100 mL*
Class-1	Excellent	10	0
Class-2	Satisfactory	1-3	0
Class-3	Suspicious	4-10	0
Class-4	Unsatisfactory	>10	0 or more

*Presence of E. *coli* immediately places the sample in Class-4, irrespective of coliform count

B. Membrane filtration method for total coliform and thermotolerant (faecal) coliforms

Principle: The method is based on the filtration of a known volume of water through a membrane filter consisting of a cellulose compound with a uniform pore diameter of 0.45 or 0.2 µm; the bacteria are retained on the surface of the membrane filter. When the membrane containing the bacteria is incubated in a sterile container at an appropriate temperature with a selective differential culture medium, characteristic colonies of coliforms/ thermotolerant coliforms develop, which can be counted directly. In contrast to the multiple-tube method, the membrane-filtration method gives as a direct count of total coliforms and thermotolerant coliforms present in a given sample of water. A comparison of the two methods, namely the multiple tube method and the membrane filtration method, depicting the relative advantages and disadvantages, is given in the table below (Table 3.3: II).

Table 3.3: II: Comparison of multiple-tube technique vs membrane filter technique

Multiple-tube method	Membrane filter method
PrincipleFermentation tubes (other suitable vessels) containing lactose broths (MacConkey) are inoculated with measured volumes of water samples; the coliform bacteria present in the water	PrincipleMeasured volume of water if filtered through a membrane (pore size 0.45 or 0.2 µm, which retains the bacteria on its surface; the membrane is then incubated on a suitable selective medium

sample multiple and are detected by formation of acid and gas. From the number of tubes inoculated and the number with a positive reaction, the most probable number (MPN) of bacteria present in the original water sample can be determined statistically.	for coliform bacteria), allowing the bacteria to multiply and form colonies. The number of colonies counted is directly related to the bacteriological content of the water sample being analysed.
Applicable to all kinds of water samples: clean, coloured or turbid, containing sewage or sewage or mud/soil particle, provided the bacteria are homogeneously distributed in the prepared test sample.	• Not suitable for turbid water· • Clay, algae, etc. prevents filtration of a sufficient volume for analysis; may produce a deposit on the membrane that could interfere with bacterial growth· • Presence of high counts of non-coliform bacteria in the sample may interfere with detection of coliforms
• Sufficiently sensitive to measure low levels of bacteria in water samples. (but a minimum time of 48 hr is required either for negative result or for a presumptive positive coliform count)	• Results are direct and obtained more quickly; coliform counts can be made in less than 24 hr.
• Not suitable for field use.	• Portable equipment, suitable for field use.

Volume of water sample or filtration: Since the filtration area is relatively small, it can support the growth of only a limited number of colonies: the optimum number is between 20 and 80, with a maximum of 200. If this figure is exceeded, very small atypical colonies or superimposed colonies may develop, or there may be growth inhibition due to overpopulation. The choice of the volume of sample to be filtered will depend on the type of water. Examples of typical volumes for membrane in Table 3.3: III.

Table 3.3: III: Typical volumes for membrane-

Sample type	Sample volume (mL)
Treated drinking-water	100
Partially treated drinking-water	10-100
Protected source water or groundwater	10-100
Surface water and water from open walls	0.1-100[a]

[a]Volumes less than 10 mL should be added to the filtration apparatus after addition of at least 10 mL of sterile diluent to ensure adequate dispersal across the surface of the membrane filter.

Equipment and glassware: In addition to the basic equipment and glassware used in the multiple-tube method, the following items are needed for the membrane-filtration technique.

- Membrane-filtration apparatus: including an electric or hand-powdered vacuum pump, a vacuum flask (e.g. an Erlenmeyer side-arm flask), and a filter support. One such commercially available filter assemble is shown below to illustrate the various components.
- Residual Petri dishes: made from glass or metal (disposable plastic Petri dishes may also be used).
- Blunt-ended forceps: For safe handling of filter membranes handle membrane filters without damage using highly polished stainless steel forcep blades with beveled, unserrated tips. Sterilise by autoclaving or by flaming.
- Reusable (autoclavable) bottles: for culture media (e.g. / 25 mL polypropylene bottles).
- A magnifying lens: with 34 or 35 magnification for examining and counting the colonies on the membrane filters. Alternatively, a colony counter (such as Quebec Colony Counter) may also be used.
- A boiling bath / pan: if filtration apparatus is to be disinfected in boiling water between analyses.
- Sterile pipettes: 1 mL and 10 mL
- A graduated cylinder: 100 mL
- In addition to the consumables needed for the MPN, the following are required:
- Membrane filters: 47 mm in diameter, with a pore diameter of 0.45 µm. Singly packed, presterilised membrane filters are very convenient. Unsterilised membrane filters can also be used, however, and should bee wrapped in paper packets in convenient numbers (depending on the number of water samples to be tested). These can then be sterilized in the autoclave and dried by rapid exhaustion of the steam.
- Nutrient absorbent pads: These are essentially filter-paper discs about 1 mm thick, with the same diameter as the membrane filters. They are available with suitable dispensers which help in rapidly dispensing large numbers of pads without individual forceps-handling and hence reduce contamination risks.
- Culture media: different types are available
- Wax pencils: for labeling Petri dishes
- Polythene bags: for wrapping Petri dishes if a dry incubator is used, to prevent drying of the sample and media

Culture media and dilution water: Various media can be used for the examination of coliform organisms by the membrane-filtration method. Of these, lactose Tergitol agar, lactose TTC Tergitol agar and membrane lauryl sulfate lactose broth may be used for coliform organisms at 35 or 37°C and for thermotolerant coliform organisms at 44°C or 44.5°C. Membrane faecal coliform (MFC) broth should be used only at 44 or 44.5°C for thermotolerant coliform counts. Although the use of all these media for the detection of presumptive coliform organisms is based on the fermentation of lactose, the characteristic reaction varies with each medium (Table 3.3: IV and 3.3: V). It is common to confirm sheen colonies from mENDO by inoculating confirmatory broth media with colonies. Your might require technician to swab the filter surface with a sterile cotton swab and use this to inoculate MacConkey, BGB and EC broths.

Table 3.3: IV: Comparison of media for the examination of Coliforms

Medium	Uses	Incubation temperature	Remarks
Lactose TTCAgar with Tergitol 7	Total or thermotolerant coliforms	18-24 hours at 35 ± 0.5°C or 37 ± 0.5°C for total coliforms and 18-24 hours at 44 ± 0.25°C for thermotolerant coliforms	Adjust pH before sterilization. Filter TTC supplement to sterilise. Tergitol supplement sterilised by autoclaving. Supplements of Tergitol and TTC to be added aseptically.
Lactose agar with Tergitol 7	Total or thermotolerant coliforms	18-24 hours at 35 ± 0.5°C for total coliforms and 18-24 hours at 44 ± 0.25°C or 44.5 ± 0.25°C for thermotolerant coliforms	Prepared plates have max. shelf-life of 10 days. Store in dark.
Membrane enrichment with Teepol broth	Total or thermotolerant coliforms	18-24 hours at 35 ± 0.5°C or 37 ± 0.5°C for total coliforms and 18-24 hours at 44 ± .25°C or 44.5 ± 0.25°C for thermotolerant coliforms	Check pH before sterilization

Membrane lauryl sulphate broth	Total or thermotolerant coliforms	18-24 hours at 35 ± 0.5°C or 37 ± 0.5°C for total coliforms and 18-24 hours at 44 ± .25°C or 44.5 ± 0.25°C for thermotolerant coliforms	Check pH before sterilization
Endo medium	Total coliforms only	35-37°C	Basic fuchsin may be a carcinogen. Also requires ethanol. Do not autoclave. Prepared medium has a shelf-life of 4 days. Store prepared medium at 4°C in the dark.
LES Endo medium	Total coliforms only	35-37°C	Basic fuchsin may be a carcinogen. Also requires ethanol. Do not autoclave. Prepared medium has a shelf-life of 2 days. Store prepared medium at 4°C in the dark.
MFC	Thermotolerant coliforms	44°C	Do not autoclave. Discard unused medium after 96 hours. Rosalic acid stock has a maximum shelf-life of 2 weeks. Check pH before sterilisation. Store prepared medium at 2-10°C.

Table 3.3: V: Colony characteristics of total coliforms and thermotolerant coliforms, following analysis by the membrane-filtration method[a]

Medium	**Colony characteristics**	
	Total coliforms at 35/37°C	Thermotolerant coliforms at 44/45.5°C

Lactose TTC* agar with Tergitol 7	Yellow, orange or brick-red colouration with yellow central halo in the medium under the membrane	As for total coliforms at 35/37°C
Lactose agar with Tergitol 7	Yellow central halo in the medium under the membrane	As for total coliforms at 35/37°C
Membrane-enriched Teepol broth	Yellow colour extending on to the membrane	As for total coliforms at 35/37°C
Membrane lauryl sulfate broth	Yellow colour extending on to the membrane	As for total coliforms at 35/37°C
Endo agar or broth	Dark red colour with golden-green metallic sheen	-
LES-Endo agar	Dark red colour with golden-green metallic sheen-	-
Membrane faecal coliform (MFC) broth		Blue colonies

*Adapted from ISO 9308-1: 1990, Detection and enumeration of coliform organisms, thermotolerant coliform organisms and presumptive Escherichia coli- part 1: Membrane filtration method.

* 2, 3, 5-Triphenyltertrazolium chloride.

Although it is possible to presence the media from the basic ingredients, this may be impractical in a small laboratory. The use of dehydrated media is therefore recommended. The media can be prepared as a broth and used together with nutrient absorption pads, or as solid agar plates. The broths may be solidified by the addition of 1.2-1.5% agar before boiling.

Procedure:

The procedure generally used is described here, but different types of filtration units and equipment exist.

a. Connect the Erlenmeyer (side-arm) flask to the vacuum source (turned off) and place the porous support in position. If an electric pump is used, it is advisable to put a

second flask between the Erlenmeyer flask and the vacuum source; this second flask acts as a water trap, and thus protects the electric pump.

b. Open a sterile Petri dish and place a sterile absorbent pad in it.

c. Add broth medium to saturate the pad; remove excess broth.

d. Place a sterile 47 mm dia. membrane filter on the porous support surface or screen, using blunt forceps sterilised by flaming. The gridded surface should be if you are using gridded membrane filter. Assemble the filter unit in the order shown in.

e. Place the upper container in position and secure it. The type of clamp used will depend on the type of equipment.

f. Pour the volume of sample chosen as optimal for the types of water into the upper container. If the test sample is less than 10 mL, at least 20 mL of sterile dilution water should be added to the top container before filtration. Apply the vacuum.

g. Take the filtration unit apart and, using the sterile forceps, place the membrane filter in the Petri dish on the pad with the grid side up. Make sure that no air bubbles are trapped between the pad and the filter.

h. Leave the Petri dish at room temperature or at 35 or 37°C for 2-4 hours, for resuscitation of stressed microbes.

i. Place the dishes in an incubator at 44 ± 0.5°C for 18-24 hours with 100% humidity. Alternatively, tight-fitting or sealed petri dishes may be placed in waterproof plastic bags for incubation.

j. Place the dishes in an incubator at 44 ± 0.5°C for 18-24 hours with 100% humidity. Alternatively, tight-fitting or sealed petri dishes may be placed in waterproof plastic bags for incubation.

k. Submerge the bags in a water-bath maintained at 44 ± 0.5°C for 18-24 hours. The plastic bags must be below the surface of the water throughout the incubation period. They can be held down by means of a suitable weight, e.g. a metal rack.

The colonies of coliform/thermotolerant coliform bacteria should be identified from their characteristics on the medium used. The number of coliforms/thermotolerant coliforms per 100 mL is then given by:

Coliform / thermotolerant coliforms per 100 mL
= [(No. of coliforms/thermotolerant coliform colonies counted) / (no. of mL of sample filtered) x 100]

Cleaning:

a. Immediately after use, disassemble the filter holder and clean its components with a sponge, hot water, and a nonabrasive cleanser. If you are cleaning the Stainless Screen Glass 47 mm Filter-Holder, the screen drops out easily when the base is

inverted. Take care not to mislay or damage the Teflon gasket that lies under the screen.

b. Use a stiff bristled brush (if available) to remove all traces of stubborn residue from recesses and orifices, but do not use the brush on the filter support screen: it can break the screen mesh and cause a ruptured filter. A test tube brush with detergent solution is helpful in cleaning the inside of the holder outlet tube. Never use steel wool or abrasive materials on any part of the holder.

c. Clean the porous glass frit of the filter support by back-flushing with warm tap water and then soaking overnight in a chromic-acid cleaning solution. Follow the soaking with another back-flushing.

d. After cleaning thoroughly, rinse the components with clean water and air dry. Do not wipe with paper or cloth, which may leave traces of fibres and lint. Autoclave the funnel and bases (if desired), wrap separately, and store for later use.

e. If filtering funnels and bases are needed for multiple analyses in a single day, they may be sanitized in boiling water for 2-3 minutes in-between samples.

C. EC-MUG Test for confirmation of E. coli

This is a simple test for confirmation of presence of E. coli in water samples and may be knitted into the Multiple Tube Fermentation (MTF) procedure, as a confirmatory test. If the lab chooses to use EC-MUG test, it would replace using BGLB and tryptone broth (indole test) at 44.5°C.

Background information: ECJ_MUG method test for the presence of E. coli bacteria. MUG stands for 4-methlumbelliferyl-ß-D-glucoronide. It is the substrate for the enzyme ß-glucoronidase. This enzyme is primarily found only in E. coli. The enzyme cleaves the MUG molecule,

Media/Equipment needed:

- EC-MUG medium in tubes (for preparation, see under the section on culture media)
- Water bath capable of maintaining temperature at 44.5°C
- Long-wave UV lamp, preferable 6-walt bulb.

Procedure: Submit all presumptive fermentation tubes/bottles showing gas/growth/or acidity within 48±3 hr of incubation, to this confirmatory test for E. coli. EC-MUG broth tubes are inoculated from positive presumptive MacConkey broth tubes/bottles. The inoculated tubes are incubated at 44.5°C for 24 hours. A positive reaction is observance of a bright blue fluorescence when the tune is subjected to long-wave (366 nm) ultraviolet (UV) light.

The use of positive (a known E. coli MUG positive culture) and negative (thermotolerant *Klebsiella pneumoniae*, MUG negative) control reactions is highly recommended. An uninoculated medium control may also be necessary to interpret the results and to avoid confusion of weak auto-fluorescence of the medium as a positive response.

Interferences: Certain brands of glass test tubes fluorece under UV light. Tubes should be examined before use. Do not use Durham tubes in the EC-MUG tubes, as gas is not relevant to the test and could interfere with the interpretation of the UV light results.

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BORON (B)

4

Scope and application

Boron is an essential element for plant growth. However, in excess of 2mg/L in irrigation water, it is deleterious to certain plants and some plants may be affected adversely by concentrations as low as 1mg/L (or even less in commercial green-houses). Drinking water rarely contain more than 1.0mg/L and generally less than 0.1mg/L concentrations considered innocuous for human consumption. Boron may occur naturally in some water or may find its way into a watercourse through cleaning compounds and industrial effluents. Seawater contains approximately 5mg/L B. The ingestion of large amounts of boron can affect the central nervous system. Protracted ingestion may result in a clinical syndrome known as "Borism".

A. Curcumin method

Principle: When a water sample containing boron is acidified and evaporated in presence of Curcumin, a red coloured product called rosocyanine is formed. This rosocyanine is extracted in organic solvent and colour is measured photometrically. This method is applicable in the range of 0.1 to 1 mg/L.

4.1 Apparatus and equipment

a. Colorimetric equipment-any one of the following is required
   1. Spectrophotometer, for use at 540 nm with minimum light path of 1cm.
   2. Filter photometer equipped with a green filter having a maximum transmittance near 540 nm with a minimum light path of 1cm.

b. Evaporating dishes 100-150mL capacity of high silica glass, platinum or other suitable material.

c. Water bath set at 55±2°C.

d. Glass stoppered volumetric flask 25mL and 50mL capacity.

e. Ion-exchange column 50 cm long by 1.3cm diameter.

4.2 Reagents and standards

a Stock boron solution: Dissolve 571.6 mg anhydrous boric acid (H_3BO_3), in distilled

water and dilute to 1000mL; 1mL = 100µgB. Because H_3BO_3 loses weight on drying at 105°C, use a reagent meeting ACS specification and keep the bottle tightly stoppered to prevent entrance of atmospheric moisture.

b. Standard Boron Solution: Dilute 10mL stock boron solution to 1000mL with distilled water, 1mL = 1µg Boron.

c. Curcumin reagent: Dissolve 40mg finely grounded Curcumin and 5.0g of oxalic acid in 80mL 95% ethyl alcohol. Add 4.2mL concentrated HCl, make up to 100mL with ethyl alcohol in a 100 mL volumetric flask and filter it, if reagent is turbid (isopropyl alcohol 95% may be used in place of ethyl alcohol). This reagent is stable for several days if stored in refrigerator.

d. Ethyl or Isopropyl alcohol, 95%

e. Reagent for removal of high hardness and cation interference:

   1. Strongly acidic cation-exchange resin.

   2. Hydrochloric acid, HCl 1:5

4.3 Sample collection, preservation and storage

Store samples in polyethylene bottles or alkali resistant, boron free glassware.

4.4 Calibration

Precaution:

Closely control such variables as volumes and concentrations of reagents as well as time and temperature of drying. Use evaporating dishes identical in size shape and composition to ensure evaporation time because increasing the time increases intensity of the resulting colour.

Pipette 0 (Blank), 0.25, 0.50, 0.75 and 1.0µg boron using micropipette in to evaporating dishes of the same type, shape and size. Add distilled water to each standard to bring total volume to 1mL. Add 4mL Curcumin reagent to each and swirl gently to mix contents thoroughly. Float dishes on water bathe set at 55±2°C and let them remain for 80 min, which is usually sufficient for complete drying and removal of HCl. Keep drying time constant for standards and samples. After dishes cool to room temperature add 10mL 95% ethyl alcohol to each dish and stir gently with a polyethylene rod to ensure complete dissolution of red coloured product. Wash contents of dish into a 25mL volumetric flask using 95% ethyl alcohol and mix thoroughly by inverting. Read absorbance of standards and samples at a wavelength of 540nm after setting reagent blank at zero absorbance. The calibration curve is linear for 0-1µg boron. Make photometric readings within 1h of drying samples.

Sample treatment:

a. Water containing 00.1 to 1 mg B/L use 1mL sample. For water containing more than 1 mg B/L make an appropriate dilution with boron free distilled water so that 1mL portion contains approximately 0.5µg B.

b. Pipette 1 mL sample or dilution in to an evaporation dish. Unless the calibration curve is determined at the same time, prepare a blank and a standard containing 0.5µg B and run in conjunction with the sample.

c. Proceed as in preparation of calibration, beginning with "Add 4.0 mL Curcumin reagent..." if the final solution is turbid through filter paper before reading absorbance.

d. Calculate boron content from calibration curve.

Visual comparison:

The method may be adapted to visual estimation of low concentration from 50-200µg/L as follows:

a. Dilute the standard Boron solution 1:3 with distilled water, 1mL = 0.20µg/L B. Pipette 0, 0.05, 1.0, 0.15 and 0.2µg B into evaporating dishes as indicated in description. At the same time add an appropriate volume of sample (1mL/portion diluted to 1mL) to an identical evaporating dish. The total boron should be between 0.05 and 2.0µg.

b. Proceed as in description beginning with "Add 4mL Curcumin reagent...."

c. Compare colour of samples with standards within 1h of drying samples.

4.5 Calculation

Use the following equation to calculate Boron concentration from absorbance reading.

$B\ mg/L = A_2 * C / A_1 * S$

Where,

A_1 = absorbance of standards
A_2 = absorbance of sample
C = µg boron in standard taken
S = mL of sample

4.6 Precision and Bias

A synthetic sample containing 240µg/L B with minimum interference was analysed in 30

Laboratories with a relative standard deviation of 22.8% and relative error of 0%. Treat blank, standards and samples in the same manner. Frequently carry our recovery procedures through known addition methods.

4.7 Interferences

NO3 concentration above 20mg/L, hardness exceeds 100mg/L $CaCO_3$ and moderate hardness results in the interferences and error in low boron range. Treatment with acidic cation can eliminate hardness interferences. Phosphate does not interfere.

4.8 Pollution prevention and waste management

Chemicals used in analysis are in micro-quantities and will be added to waste in very dilute form. It will not necessarily cause any problems; however routine waste management practices should be followed.

B. Carmine method

Principle: In the presence of Boron, a solution of carmine or carmine acid in concentrated sulphuric acid changes from bright red to bluish red or blue, depending on the concentration of boron present.

4.9 Apparatus and equipment

Colorimetric equipment-any one of the following is required

1. Spectrophotometer, for use at 585nm with minimum light path of 1cm.
2. Filter photometer equipped with a green filter having a maximum transmittance near 540nm with a minimum light path of 1cm.

4.10 Reagent and standards

a Hydrochloric acid 1:11 (1+11)

b. Standard Boron Solution: Dilute 10mL stock boron solution to 1000mL with distilled water, 1mL = 1µg Boron.

c. Sulphuric acid, conc. H_2SO_4

d. Carmine reagent: Dissolve 920mg carmine N.F. 40, or carmine acid, in 1L conc. H_2SO_4. (If unable to zero spectrophotometer dilute carmine 1+1 with conc. H_2SO_4 to replace above reagent).

4.11 Sample collection, preservation and storage

Store samples in polyethylene bottles or alkali resistant, boron free glassware.

4.12 Calibration

a. Prepare a series of boron standard solutions (100, 250, 500, 750 and 1000µg) in 100mL with distilled water. Pipette 2mL each standard solution into small flask or 30mL test tube.
b. Treat blank and Calibration standards exactly as sample.

4.13 Procedure

Preliminary sample treatment:

a. If the sample contains less than 1mg/L B, pipette a portion containing 2 to 20µg B into a platinum dish, make alkaline with 1N NaOH slightly in excess and evaporate to dryness on a steam or hot water bath.
b. If necessary, destroy any organic matter by ignition at 500 to 550 C. Acidify cooled residue with 2.5mL of 1+11 HCl and triturate with rubber policeman to dissolve.
c. Centrifuge if necessary to obtain a clear solution.
d. Pipette out 2mL clear concentrate into a small flask or 30mL test tube. Treat reagent blank identically.

Colour Development:

a. To the sample, blank and standard add 2 drops (0.1mL) conc. HCl, carefully introduce 10mL conc. H_2SO_4, mix and let it cool to room temperature.
b Add 10mL carmine reagent, mix well, and after 45 to 60 min, measure absorbance at 585nm in a cell of 1cm path length, using the blank as reference.

4.14 Calculations

B mg/L = µg B (in approximately, 22mL final volume) / mL of sample

4.15 Precision and Bias

A synthetic sample containing 180µg/L B with minimum interferences was analysed in 30 laboratories with a relative standard deviation of 35.5% and relative error of 0.6%/ Treat blank, standards and samples in the same manner. Frequently carry out recovery procedures through known addition methods.

4.16 Interferences

The ions commonly found in water and wastewater do not interfere.

4.17 Pollution prevention and waste management

Chemicals used in analysis are in micro-quantities and will be added to waste in very dilute form. It will not necessarily cause any problems; however routine waste management practices should be followed.

4.18 Bibliography

Standard Method for the Examination of Water and Wastewater, APHA, AWWA and WEF, 21st Edition. 2005.

PHENOLS

5

Introduction:

The presence of organic compounds in water and wastewater is a matter of increasing concern to the water industry, environmentalists and general public. The impact of such compounds may differ. For example, biologically oxidisable compounds will deplete oxygen in the water body and petroleum products. Oil and grease will adversely affect biological activity. Phenols, detergents and other organic materials may be toxic to phyto and zoo-plankton beyond certain levels. Organic compounds may enter water environment through human waste disposal and industrial discharges. A variety of sensitive instruments have been developed to quantify the organic pollutants. BOD, COD, TOC, oil and grease, phenols, detergents-surfactants, and TFPs, are certain demand parameters considered to evaluate levels of contamination.

Phenols are defined as hydroxyl derivatives of benzene, and its condensed nuclei occur in domestic and industrial wastewaters, natural wastes and potable water supplies. Odoriferous and objectionable tasting chlorophenols are formed as a result of chlorination of water containing phenol. Phenols may be present in raw water owing to the discharge of wastewaters from coke distillation plants, the petrochemical industry and numerous other industries where phenols serve as intermediates. They are also present in municipal wastewaters.

Presence of phenols may lead to objectionable taste in chlorinated drinking water and hence its monitoring is essential. Phenols can be removed from drinking water by super-chlorination (chlorine-dioxide or chloramines treatment) ozonation and activated carbon adsorption.

Methods

4-aminoantipyrine colorimetric method is generally used for the determination of phenol, ortho and meta-substituted phenols and under proper pH conditions, phenol in which the substitution is a carboxyl, halogen, methoxyl or suphonic acid group. It does not determine those para substituted phenols where the substituted group is an alkyl, aryl, nitro, benzoyl, nitroso or aldehyde group. Chloroform extraction and direct photometric methods used for the determination of phenols are detailed hereunder.

A. Chloroform extraction method

The steam distillable phenols react with 4-aminoantipyrine at a pH of 7.9 in presence of potassium ferricyanide to form a coloured antipyrine dye. The dye is extracted from aqueous solution with chloroform and the intensity is measured at 460 nm. This method is applicable in the concentration range of 1 µg/L to 250 µg/L with a sensitivity of µg/L.

5.2 Apparatus and equipment

a. Distillation assembly: All glass consisting of 1 L pyrex distilling apparatus with Graham condenser.

b. Spectrophotometer for use at 460 nm

c. pH meter

d. Separatory funnels: 1000 mL Squibb form, with ground glass stoppers and Teflon stopcocks

e. Beakers: 1L.

5.3 Reagent and standards

a. Phosphoric acid (1+9): Dilute 10 mL 5% H_3PO_4 to 100 mL with distilled water.

b. Methyl orange indicator: Dissolve 0.5 g methyl orange in one litre distilled water.

c. Sulphuric acid 1N: Dilute 28 mL of conc. H_2SO_4 to 1 L with distilled water.

d. Sodium chloride: NaCl, solid crystals.

e. Chloroform or ethyl ether: $CHCl_3$, AR grade.

f. Sodium hydroxide, 2.5N: Dissolve 10 g NaOH in 100 mL distilled water.

g. Stock phenol solution: Dissolve 1 g phenol in freshly boiled and cooled distilled water and dilute to 1 litre. Standardise the stock phenol solution. 1 mL = 1mg phenol.

h. Intermediate phenol solution: Take 10 mL or appropriate volume of stock phenol solution in 1 litre volumetric flask and dilute to the mark with freshly boiled and cooled distilled water as to get 1 mL = 10 µg phenol.

i. Standard phenol solution: Dilute 50 mL intermediate phenol solution to 500 mL with freshly boiled and cooled distilled water. This solution should be prepared within two hours of use. 1 mL = 1 mg phenol.

j. Bromate bromide solution (0.1N): Dissolve 2.784 g anhydrous $KBrO_3$ in water, add 10 g KBr crystals, dissolve and dilute to 1000 mL with distilled water.

k. Hydrochloric acid: HCl conc.

l. Standard sodium thiosulphate (0.025N): Dissolve 6.205 g $Na_2S_2O_3.5H_2O$ in distilled water and dilute to 1000 mL.

m. Starch solution: Dissolve 2 g laboratory grade soluble starch in 100 mL hot distilled water. Prepare this solution daily.

n. Ammonium hydroxide (0.5N): Dilute 35 mL fresh concentrated NH_4OH to 1000 mL with distilled water.

o. Phosphate buffer solution: Dissolve 104.5 g K_2HPO_4 and 72.3 g KH_2PO_4 in distilled water and dilute to 1 litre, the pH of this solution should be 6.8.

p. 4-Aminoantipyrine solution: Dissolve 2.0 g 4-aminoantipyrine in distilled water and dilute to 100 mL. Prepare this solution daily.

q. Potassium ferricyanide solution: Dissolve 8.0g $K_3Fe(CN)_6$ in water and dilute to 100 mL. Filter if necessary and store in a brown glass bottle.

r. Sodium sulphate: Anhydrous Na_2SO_4, granular.

s. Potassium iodide: Kl, solid crystals.

5.4 Sample collection, preservation and storage

After collection of sample, analyse within 4 hours, if not, preserve and store by acidifying to pH 4 with H_3PO_4 under cool environment (5°C). Add 1 g $CuSO_4.5H_2O$/L of sample to inhibit biological degradation. Analyse preserved samples within 24 hours.

5.5 Calibration

Stock phenol solution: Take 100 mL distilled water in a 500 mL glass stoppered conical flask or iodine flask. Add 50 mL stock phenol solution and 10 mL 0.1N bromate-bromide solution followed by 1 mL concentrated HCl and swirl gently. If brown colour of free bromine does not persist, add 10.0 mL portions bromate-bromide solution until it does. Put the stopper tightly and keep it for 10 minutes. Then add 1 g Kl, keep flask stoppered, swirl and again keep it for some time. Prepare a blank in the same manner using distilled water and 10 mL 0.1N bromate-bromide solutions. Titrate blank and sample with 0.025 N sodium thiosulphate using starch as an indicator. Calculate the concentration of phenol solution as follows:

Mg/L phenol = 7.842 [(A x B) c]
Where:
A = mL thiosulphate for blank
B = mL bromate-bromide solution used for sample divided by 10
C = mL thiosulphate used for sample

5.6 Procedure

I. Distillation:

A. Measure 500 mL sample into a beaker, add 50 mL phenol-free distilled water, lower the pH to 4.0 with H_3PO_4 solution using methyl orange as an indicator. Add 5 mL $CuSO_4$ solution. Transfer to distillation flask and collect 500 mL distillate using measuring cylinder as receiver. If the distillate is turbid repeat the same procedure as above. Omit addition of H_3PO_4 and $CuSO_4$ if the preserved sample is used.

B. Take 500 mL original sample. Make it acidic with 1 N H_2SO_4 using methyl orange as an indicator. Transfer into a separating funnel and add 150 g NaCl. Shake with five increment of chloroform, using 40 mL in the first increment and 25 mL in each of the following increments. Transfer the chloroform layer to another separatory funnel and shake with three successive increments of 2.5 N NaOH solution using 4.0 mL in the first increment and 3.0 mL in each of the next two increments. Combine the alkaline extracts. Heat on water bath until the chloroform has been removed. Cool and dilute to 500 mL with distilled water and proceed for distillation as in (A).

II. Extraction and colour development:

a. Take 500 mL of the distillate, or a suitable portion containing for more than 50 mg phenol and dilute to 500 mL in 1 litre beaker.

b. Take 500 mL distilled water blank and a series of 500 mL phenol standards containing 5, 10, 20, 30, 40 and 50 µg phenol, in respective beakers.

c. Add 12 mL 0.5 N NH_4OH solution and adjust the pH of each to 7.9 ± 0.1 with phosphate buffer. About 10 mL phosphate buffer is required. Transfer to 1 litre separating funnel, add 3.0 mL 4-aminoantipyrine solution in each separatory funnel, mix well and add 3.0 mL potassium ferricyanide, again mix well and let the colour develop for 15 min.

d. Add 25 mL chloroform in each separatory funnel and shake at least 10 times, let the $CHCl_3$ settle again. Filter each $CHCl_3$ extract through filter paper containing 5 g layer of anhydrous Na_2SO_4.

e. Collect dried extract clean cells and measure the absorbance of sample and standard against the blank at 460 nm. Plot absorbance against mg phenol concentration and draw a calibration curve. Estimate sample phenol content from photometric reading by using a calibration curve.

5.7 Calculations

Use of calibration curve,

$\mu g/L, \text{phenol} = [(A/B) \times 1000]$

Where:

A = µg phenol in sample (estimated from calibration curve)
B = mL original sample

5.8 Precision and Bias

The phenolic value varies with types of phenols within given sample. Since 'phenol' value is based on C_6H_5OH, the method is regarded only as an approximation. Therefore, it is impossible to express accuracy of the method. Treat blank, standard and samples in the same manner.

5.9 Interference

Phenol decomposing bacteria, oxidising reducing substances and alkaline pH value may interfere. All interferences are eliminated or reduced to minimum, if sample is appropriately preserved, stored and distilled. Biological degradation is inhibited by addition of $CuSO_4$. Addition of H_3PO_4 ensures presence of copper ion and eliminates chemical changes due to alkaline conditions.

5.10 Safety

Hazardous chemicals like chloroform, phosphoric acid, potassium ferricyanide and phenol should be handled with required precautions.

5.11 Pollution prevention and waste minimization

Precautions are needed for proper disposal of processed samples containing hazardous chemicals added during estimation procedure.

B. Direct photometric method

5.12 Principle

Steam distillable phenolic compounds react with 4-aminoantipyrine at pH 7.9 ± 1 in presence of potassium dye. The intensity of the coloured dye is measured at 500 nm which is proportional to the concentration of phenol present in the sample.

This method is less sensitive than the previous one. The minimum detectable quantity is 10mg phenol when a 5 cm cell and 100 mL distillate are used.

5.13 Apparatus and equipment

Spectrophotometer for use at 500nm and pH meter.

5.14 Reagents and standards

As prepared in the chloroform extraction method.

5.15 Sample collection, preservation and storage

After collection of sample, analyse it within 4 h. If not, preserve and store by acidifying to pH 4 with H_3PO_4 under cool environment (5°C). Add 1 g $CuSO_4$ $5H_2O$/L of sample to inhibit biological degradation. Analyse preserved samples within 24 hours.

5.16 Calibration

Refer section5.5.

5.17 Procedure

I. Distillation: Refer Section 5.6,1

II. Absorbance:

a. Take 100 mL distillate or an aliquot of the distillate containing not more than 0.5 mg phenol and dilute to 100 mL in a 250 mL beaker.

b. Prepare a 100 mL distilled water blank and a series of 100 mL phenol standards containing 0.1, 0.2, 0.3, 0.4 and 0.5 mg phenol.

c. Add 2.5 mL 0.5N NH_4OH solution in each and adjust the pH to 7.9 ± 0.1 with phosphate buffer.

d. Add 1 mL 4-aminoantipyrine solution and 1 mL $K_3Fe(CN)_6$ solution in each flaks and mix well.

e. Measure the absorbance of each after 15 minutes against blank at 500 nm.

f. Plot absorbance against mg phenol concentration. Draw a calibration curve. Estimate sample phenol from photometer reading by using a calibration curve.

5.18 Calculations

Use of calibration curve,

µg/L, phenol = [(A/B) x 1000]

Where:
A = mg phenol in sample (estimated from calibration curve)

B = mL original sample

5.19 Precision and Bias

The phenolic value varies with types of phenols within given sample. Since 'phenol' value is based on C_6H_5OH, the method is regarded only as an approximation, and it is impossible to express accuracy of the method. Treat blank, standards and samples in the same manner.

5.20 Interferences

Interferences are reduced by using the distillate from the preliminary distillation procedure.

5.21 Safety

Hazardous chemicals like chloroform, phosphoric acid, potassium ferricyanide, phenol, etc. should be handled with required precautions.

5.22 Pollution prevention and waste minimization

Precautions are needed for proper disposal of processed samples containing hazardous chemicals added during the estimation procedure.

5.23 Bibliography

1. Standard Methods for the Examination of Water and Wastewater; APHA, AWWA and WEF, 21st Edition, 2005.

6 pH Value

Introduction:

The pH of a solution is measured as negative logarithm of hydrogen ion concentration. At a given temperature, the intensity of the acidic or basic character of a solution is indicated by pH or hydrogen ion concentration. pH values from 0 to 7 are diminishing acidic, 7 to 14 increasingly alkaline and 7 is neutral.

Measurement of pH is one of the most important and frequently used tests, as every phase of water and wastewater treatment and waste quality management is pH dependent.

The pH of natural water usually lies in the range of 4 to 9 and mostly it is slightly basic because of the presence of bicarbonates and carbonates of alkali and alkaline earth metals. pH value is governed largely by the carbon dioxide/ bicarbonate/ carbonate equilibrium. It may be affected by humic substances, by changes in the carbonate equilibriums due to the bioactivity of plants and in some cases by hydrolysable salts. The effect of pH on the chemical and biological properties of liquid makes its determination very important. It is used in several calculations in analytical work and its adjustment to an appropriate value is absolutely necessary in many of analytical procedures.

In dilute solution, the hydrogen ion activity is approximately equal to the concentration of hydrogen ion. Pure water is very slightly ionized and at equilibrium the ionic product is:

$[H^+][OH^-] = K = 1.0 \times 10^{-14}$ at 25°C

OR

$[H^+] = [OH^-] = 1.005 \times 10^{-7}$

A logarithmic form is,

$[-\log_{10}(H^+)]\,[-\log_{10}(OH^-)]$

Or

$pH + pOH = pK_w$

A. Electrometric method

From the above equilibrium it is clear that the pH scale for an aqueous solution lies between 0 and 14. The pH determination is usually done by electrometric method, which is the most accurate one, and free from interferences. The Colorimetric indicator methods can be used only if approximate pH values are required.

6.1 Principle

The pH is determined by measurement of the electromotive force (emf) of a cell comprising of an indicator electrode (an electrode responsive to hydrogen ions such as glass electrode) immersed in the test solution and a reference electrode (usually a calomel electrode). Contact is achieved by means of a liquid junction, which forms a part of the reference electrode. The emf of this cell is measured with pH meter.

Since the pH is defined operationally on a potentiometric scale, the measuring instrument is also calibrated potentiometrically with an indicating (glass) electrode and a reference electrode using standard buffers having assigned pH value so that

$$pH_B = -\log_{10}[H^+]$$

where pH_B = assigned pH of standard buffer.

The operational pH scale is used to measure sample pH and is defined as:

$$pHs = pH_B + F(E_s - E_B)/2.303\,RT$$

where,

pHs = potentiometrically measured sample PH

F = Faraday 9.649×10^4 coulomb/mole
E_s = Sample emf V
E_B = Buffer emf V
R = Gas constant 1.987 cal deg-1 mole-1
T = absolute temperature, °K

6.2 Apparatus and equipment

a. pH meter: Consisting of potentiometer, a glass electrode, a reference electrode and a temperature compensating device. A balanced circuit is completed through potentiometer when the electrodes are immersed in the test solution. Many pH meters are capable of reading pH or millivolt.

b. Reference electrode: Consisting of a half cell that provides a standard electrode

potential. Generally calomel, silver-silver chloride electrodes are used as reference electrode.

c. Sensor (glass) electrode: Several types of glass electrodes are available. The glass electrode consists essentially of a very thick walled glass bulb, made of low melting point glass of high electrical conductivity, blown at the end of a glass tube. This bulb contains an electrode, which has a constant potential, e.g. a platinum wire inserted in a solution of H^+ hydrochloric acid saturated with quinhydrone. The bulb is placed in the liquid where pH is to be determined.

d. Beakers: Preferably use polyethylene or TFE beakers.

e. Stirrer: Use a magnetic TFE coated stirring bar.

6.3 Reagents and standards

a. pH 4 buffer solution: Dissolve 10.12g potassium hydrogen phthalate, $KHC_8H_4O_9$ in distilled water. Dilute to 1L.

b. pH 7 buffer solution: Dissolve 1.361g anhydrous potassium dihydrogen phosphate, KH2PO4, and 1.42g anhydrous disodium hydrogen phosphate, Na_2HPO_4, which have been dried at 110°C. Use distilled water which has been boiled and cooled. Dilute to 1L.

c. pH 9.2 buffer solution: Dissolve 3.81gm borax, $Na_2B_4O_7.10H_2O$ in distilled water, which has been previously boiled and cooled. Dilute to 1L.

6.4 Sample collection, preservation and storage

The procedure is given in detail in chapter 1.

6.5 Calibration

Before use, remove the electrodes from the water and rinse with distilled or demineralised water. Dry the electrodes by gentle wiping with a soft tissue. Calibrate the electrode system against standard buffer solution of known pH. Because buffer solution may deteriorate as a result of mould growth or contamination, prepare fresh as needed for work or use readily available pH buffers. Use distilled water a conductivity of less than 2μ siemens at 25°C and distilled and pH 5.6 to 6.0 for the preparation of all standard solutions. For routine analysis, commercially available buffer tablets, powders or solutions of tested quality also are permissible. Buffer having pH 4.0, 7.0 and 9.2 are available. In preparing buffer solutions from solid salts, dissolve all the material; otherwise, the pH calibration will be incorrect. Prepare and calibrate the electrode system with buffer solutions with pH approximating that of the sample, to minimise error resulting from nonlinear response of the electrode.

6.6 Procedure

a. Before use, remove electrodes from storage solutions (recommended by manufacturer) and rinse with distilled water.

b. Dry electrodes by gently blotting with a soft tissue paper, standardise instrument with electrodes immersed in a buffer solution within 2 pH units of sample pH.

c. Remove electrodes from buffer, rinse thoroughly with distilled water and blot dry.

d. Immerse in a second buffer below pH 10, approximately 3 pH units different from the first, the reading should be within 0.1 unit for the pH of second buffer. (If the meter response shows a difference greater than 0.1 pH unit from expected value, look for trouble with the electrodes or pH meter)

e. For samples analysis, establish equilibrium between electrodes and sample by stirring sample to ensure homogeneity and measure pH.

f. For buffered samples (or those with high ionic strength), condition the electrodes after cleaning by dipping them into the same sample, and read pH.

g. With poorly buffered solutions (dilute), equilibrate electrodes by immersing in three or four successive portions of samples. Take a fresh sample and record the pH.

6.7 Calculation

The pH value is obtained directly from the instrument.

6.8 Precision and Bias

The precision and accuracy attainable with a given pH meter will depend on the type and condition of the instrument and the care used in standardisation an operation. Guard against possible erratic results arising from mechanical or electrical failures-weak batteries, damaged electrodes, plugged liquid junctions, and fouling of electrodes. With proper care, a precision of ±0.02 pH unit and an accuracy of ±0.05 pH unit can be achieved. However, ±0.1 pH unite represents the limit of accuracy under normal conditions. For this reason, report pH values to the nearest 0.1 pH unit.

6.9 Interferences

The pH can be measured either colorimetrically or electrometrically. The colorimetric method is less expensive but suffers from interference from colour turbidity, salinity, colloidal matter and various oxidants and reductants. The indicators are subjected to deterioration as they are colour standards with which pH is compared. More over no single indicator encompasses the pH range of interest in water.

The glass electrode method (electrometric) is a standard technique free from interferences in natural water except for a sodium error associated with pH above 10.

6.10 References

1. American Water Works Association, (1964). Simplified Procedures for Water Examination, Manual Mi2, AWWA, New York.
2. Standard Methods for the Examination of Water and Wastewater; APHA, AWWA, and WEF, 21st Edition, 2005.

COLOUR

7

7.1 Principle

The method is useful in the field by comparing the colour of sample with a comparator. When viewed by transmitted light through a depth of several feet, pure water exhibits a light blue colour which may be altered by the presence of organic matter to greenish blue, green, greenish yellow, yellow or brown. Colour is removed to make water suitable for general and industrial applications. Coloured industrial wastewater may require colour removal before discharge into water sources.

The visual comparison method is applicable to nearly all samples of potable water. Pollution by certain industrial wastes may produce unusual colours that cannot be matched. In such a case, an instrumental method is useful. Colour expressed in terms of Pt/Co standard unit.

7.2 Sample collection, preservation and storage

Collect representative samples in clean glassware. Make the colour determination at the earliest, because biological or physical changes occurring in the storage may affect the colour. With naturally coloured water changes invariably lead to poor results.

7.3 Calibration

The colour of the sample is compared with the glass comparator and colourless distilled water or prepare a standard (5-70 units) by diluting the working standard for comparison with the sample collected.

7.4 Method performance

In coloured sample, it is impossible to match the colour with standard. In these cases the yellow colour of the sample should be determined.

7.5 Pollution prevention and waste management

There is little need for waste management as no large volumes of solvents of hazardous chemicals are used. However, laboratory waste management practices must be followed consistently with all applicable rules and regulations, and protect the water and land by

minimising and controlling all releases from bench operations. Also, compliance is required with any sewage discharge permits and regulations, particularly the hazardous waste identification rules and land disposal restrictions.

8

Dissolved Oxygen (DO)

Introduction:

All living organisms are dependent upon oxygen in one form or the other to maintain the metabolic processes that produce energy for growth and reproduction. Aerobic processes are of great interest, which need free oxygen for wastewater treatment. Dissolved Oxygen (DO) is also important in precipitation and dissolution of inorganic substances in water.

DO levels in natural waters and wastewaters depend on physical, chemical and biological activities in water body. The solubility of atmospheric oxygen in fresh water ranges from 14.6mg/L at 0°C to about 7.0mg/L at 35°C under normal atmospheric pressure. Since it is poorly soluble gas, its solubility directly varies with the atmospheric pressure at any given temperature.

Analysis of DO is a key test in water pollution control and wastewater treatment processes. The following illustrations reveal importance of DO as a parameter:

- It is necessary to know DO levels to assess quality of raw water and to keep a check on stream pollution.
- In wastewaters, dissolved oxygen is the factor that determines whether the biological changes are brought out by aerobic or anaerobic organisms.
- DO test is the basis of BOD test which is an important parameter to evaluate pollution potential of wastes.
- DO is necessary for all aerobic biological wastewater treatment processes.
- Oxygen is an important factor in corrosion. DO test is used to control the amount of oxygen in boiler feed waters either by chemical or physical methods.

A. The Winkler method with azide modification

8.1 Principle

Oxygen present in sample rapidly oxidises the dispersed divalent manganous hydroxide to its higher valency, which is precipitated as a brown hydrated oxide after the addition of NaOH/KOH and KI. Upon acidification, manganese reverts to divalent state and liberates iodine from KI equivalent to the original DO content. The liberated iodine is titrated against

$Na_2S_2O_3$ (N/40) using starch as an indicator. The chemical reactions involved in the method are given below:

1. $MnSO_4 + 2KOH \rightarrow Mn(OH)_2 + K_2SO_4$ (white ppt)
2. $2\,Mn(OH)_2 + O_2 \rightarrow 2\,MnO(OH)_2$ (Brown ppt)
3. $MnO(OH)_2 + 2H_2SO_4)_2 + 3H_2O$
4. $Mn(SO_4)_2 + 2\,KI \rightarrow MnSO_4 + K_2SO_4 + I2$
5. $2Na_2S_2O_3.5H_2O + I_2 \rightarrow Na_2S_4O_6 + 2NaCl + 10H_2O$
6. $2NaN_3 + H_2SO_4 \rightarrow 2HN_3 + Na_2SO_4$
7. $HNO_2 + HN_3 \rightarrow N_2 + N_2O + H_2O$

8.2 Apparatus and equipment

a. BOD bottles, capacity 300mL

b. Sampling device for collection of samples

8.3 Reagents

a. Manganese sulphate: Dissolve 480g $MnSO_4.4H_2O$ or 400g $MnSO_4.2H_2O$ in distilled to 1000mL. Filter if necessary. This solution should not give colour with starch when added to an acidified solution of KI.

b. Alkali iodide-azide reagent.

1. For saturated or less than saturated samples: Dissolve 500g NaOH (or 700g KOH) and 150g KI (or 135g NaI) in distilled water and dilute to 1000mL. Add 10g sodium azide, NaN_3 dissolved in 40mL distilled water. This solution should not give colour with starch solution when diluted and acidified.
2. For supersaturated samples: Dissolve 10g NaN_3 in 500mL distilled water. Add 480g NaOH and 750g NaI and stir to dissolve the contents.

   Cautions: Do not acidify this solution because toxic hydrozoic acid fumes may be produced.

c. Sulphuric acid: H_2SO_4, conc., 1mL is equivalent to about 3mL alkali-iodide-azide reagent.

d. Starch indicator: Prepare paste or solution of 2.0g of soluble starch powder and 0.2g salicylic acid as preservative in distilled water. Pour this solution in 100mL boiling distilled water. Continue boiling for a few minutes, cool and then use.

e. Stock sodium thiosulphate, 0.1N: Dissolve 24.82g $Na_2S_2O_3.5H_2O$ in distilled water. Preserve by adding 0.4g solid NaOH or 1.5mL of 6N NaOH and dilute to 1000mL.

a. Standard sodium thiosulphate, 0.025N: Dilute 250mL stock $Na_2S_2O_3$ solution to 1000mL with freshly boiled and cooled distilled water. Add preservative before making up the volume. (This should be standardised with standard dichromate solution for each set of titrations).

8.4 Sample collection, preservation and storage

Sampling for dissolved oxygen depends upon the source and method of analysis. While sampling, sample should not remain in contact with air or should not be agitated. These conditions can cause severe change in gaseous content. Sampling from any depth in streams, lakes or reservoir needs special precautions to eliminate changes in pressure and temperature. There are specific procedures and equipment developed for sampling water under pressure and unconfined water.

Sample should be collected in narrow mouth glass BOD bottles of 300mL capacity. Let the bottle overflow for some time and then stopper the bottle so that no air bubbles could form.

The DO determination should be carried out immediately after sampling.

8.5 Procedure

1. Collect sample in a BOD bottle using Do sampler.
2. Add 1mL $MnSO_4$ followed by 1mL of alkali-iodide-azide reagent to a sample collected in 250 to 300mL bottle up to the brim. The tip of the pipette should be below the liquid level while adding these reagents. Stopper immediately. Rinse the pipettes before putting them to reagent bottles.
3. Mix well by inverting the bottle 2-3 times and allow the precipitate to settle leaving 150mL clear supernatant. The precipitate is white if the sample is devoid of oxygen, and becomes increasingly brown with rising oxygen content.
4. At this stage, add 1mL conc. H_2SO_4. Replace the stopper and mix well till precipitate goes into solution.
5. Take 201mL of this solution in a conical flask and titrate against standard $Na_2S_2O_3$ solution using starch (2mL) as an indicator. When 1mL $MnSO_4$ followed by 1mL alkali-iodide-azide reagent is added to the samples as in (2) above, 2mL of original sample is lost. Therefore 201mL is taken for titration which will correspond to 200mL of original sample.

   200 x 300/ (300-1) = 201mL

8.6 Calculation

1mL of 0.025N $Na_2S_2O_3$ = 0.2mg of O_2

DO in mg/L = (0.2 x 1000) x (0.025N) ml of thiosulphate / 200

8.7 Modification in Winkler method

Following modifications are suggested to compensate for various interferences.

I. Alsterberg azide modification: The method outlined earlier is known as Azide Modification of Winkler method and also as Alstererg Azide modification. The reagent NaOH + KI + NaN_3 can be used in the method to eliminate interference caused by NO_2. This also reduces interference due to higher concentration of ferric ions.

II. Redeal Stewart Modification: This modification is used when the samples contain ferrous ions. Add 0.7mL conc. H_2SO_4 followed by 1mL 0.63% $KMnO_4$ immediately after sample collection in the BOD bottle. If ferric ions are present in large concentration, add 1mL of 40% KF solution. Remove excess $KMnO_4$ as excess oxalate produced negative error.

III. Alum flocculation Modification: Samples containing high suspended matter consume appreciable amount of iodine in acidic condition. Therefore, the samples are treated as follows:

Add 10mL of 10% alum solution followed by 1-2mL NH_4OH to 1000mL of the sample. Allow to settle for 10 minutes and siphon the clear supernatant for DO estimation.

IV Copper sulphate-sulphamic acid flocculation modification: Activated sludge contains biological flocs having high demand for O_2. Samples from such treatment plants are fixed by adding 10mL copper sulphate-sulphamic acid reagent to 1000mL of the sample. The reagent is prepared by adding 32g $CuSO_4$ in 500mL distilled water + 25mL acetic acid.

V Alkaline Hypochlorite Modification: To overcome interference of complex sulphur compounds (wastewater from sulphite pulp industry), this modification is used.

Pre-treatment with alkaline hypochlorite solution converts polythionates to sulphates and free sulphur. Excess of hypochlorite is destroyed by addition of KI and sodium sulphite.

8.8 Calibration

Select the test is titrimetric, the calibration of standard is not relevant.

8.9 Precision and Bias

The minimum detectable limit is 0.1mg/L for reproducible and accurate results. It is better to repeat the test. Check the quality of reagents. Use AR Grade chemicals. Analyse the samples in duplicate for quality assurance.

8.10 Interferences

Ferrous ion, ferric ion, nitrite, microbial mass and high suspended solids constitute the main sources of interferences. Modifications to reduce these interferences are described in the procedure.

8.11 Pollution prevention and waste management

The acidic liquid samples after titration of dissolved oxygen should be flushed with sufficient water for adequate dilution.

B. Membrane electrode method

Various modifications for the iodometric method have been developed to eliminate or minimise effects of interferences; nevertheless, the method is still not applicable to a variety of industrial and domestic wastewaters. Moreover, the iodometric method is not suited for field testing and cannot be adopted easily for continuous monitoring or for DO determinations in-situ. With membrane covered electrode systems, these problems are minimised, because the sensing element is protected by an oxygen permeable plastic membrane that serves as a diffusion barrier against impurities.

These electrodes are especially useful for taking dissolved oxygen profiles of reservoirs and streams. The electrodes can be lowered to micrometer located at the surface. They can also be suspended in biological waste treatment tanks to monitor DO level at any point. In laboratory investigations, membrane electrodes have been used for continuous DO analysis in bacterial cultures, including the BOD test. These electrodes provide an excellent method for DO analysis in polluted wastes, highly coloured waters, and strong waste effluents.

Oxygen sensitive membrane electrode of the polarographic or galvanic type are composed of two solid metal electrodes in contact with supporting electrolyte separated from the test solution by a sensitive membrane (polyethylene and fluorocarbon membranes are commonly used).

Membrane electrodes are commercially available in some variety. In all these instruments, the 'diffusion current' is linearly proportional to the concentration of molecular oxygen. The current can be converted to concentration units easily by a number of calibration procedures.

For calibration of the instrument, follow manufacturer's procedure exactly to obtain designed precision and accuracy. Usually these electrodes are calibrated by reading against air or a sample of known Do concentration (determined by iodometric method) as well as sample with zero DO (add excess of $Na_2S_2O_3$ and a trace of $CoCl_2$ to bring DO to zero). Preferably calibrate with samples of water under test. Avoid an iodometric where interfering substances are suspected.

Membrane electrodes exhibit a relatively high temperature coefficient largely due to changes in the membrane permeability. Thus, either accurate temperature measurements may be made along with DO measurements so that a correction can be applied or instruments which are equipped with a thermistor or other device to compensate automatically for temperature changes must be used.

To use the Do membrane electrode in estuaries waters or in wastewater with varying ionic strength, correction should be made for effect of salting out on electrode sensitivity. This effect is particularly significant for large changes in salt content. Calibrate with a sample of clean water containing the same salt content as the sample. Add concentrated solution of KCl to produce the sample specific conductance as that in the sample.

8.12 References

1. Standard Methods for the Examination of Water and Wastewater; APHA, AWWA and WEF, 21st EDITION, 2005.
2. Chemistry for Environmental Engineering, 4th Edition Clair N. Sawyer, Perrg Z Mc. Carty, Gener F. Parkin.

Hardness

9

Introduction

Water hardness is a traditional measure of the capacity of water to precipitate soap. Hardness of water is not a specific constituent but is a variable and complex mixture of cations and anions. It is caused by dissolved polyvalent metallic ions. In fresh water, the principal hardness causing ions are calcium and magnesium which precipitate soap. Other polyvalent cations also may precipitate soap, but often are in complex form, frequently with organic constituents, and their role in water hardness may be minimal and difficult to define. Total hardness is defined as the sum of the calcium and magnesium concentration, both expressed as $CaCO_3$, in mg/L. The degree of hardness of drinking water has been classified in terms of the equivalent $CaCO_3$ concentration as follows:

Soft	0-60 mg/L
Medium	60-120mg/L
Hard	120-180mg/L
Very hard	>180mg/L

Although hardness is caused by cation, it may also be discussed in terms of carbonate (temporary) and non-carbonate (permanent) hardness. Carbonate hardness refers to the amount of carbonates and bicarbonates in solution that can be removed or precipitated by boiling. This type of hardness is responsible for the deposition of scale in hot water pipes and kettles. When total hardness is numerically greater then that of total alkalinity expressed as $CaCO_3$, the amount of hardness equivalent to alkalinity is called carbonate hardness[1]. When the hardness is numerically equal to less than total alkalinity, all hardness is carbonate hardness. The amount of hardness in excess of total alkalinity expressed as $CaCO_3$ is non-carbonate hardness. Non-carbonate hardness is caused by the association of the hardness-causing cation with sulphate, chloride or nitrate and is referred to as "permanent hardness". This type of hardness cannot be removed by boiling.

Public acceptability of the degree may vary considerably from community depending on local conditions, and the association. The taste threshold for magnesium is less than that for cation.

A EDTA titration method

9.1 Principle

Hardness is determined by the EDTA method in alkaline condition; EDTA and its sodium salts from a soluble chelated complex with certain metal ions. Calcium and Magnesium ions develop wine red colour with Eriochrome black T in aqueous solution at pH 10.0 ± 0.1. When EDTA is added as a titrant, Calcium and Magnesium divalent ions get complexed resulting in sharp change from wine red to blue which indicates end-point of the titration. Magnesium ion must be present to yield satisfactory point of the titration. Hence, a small amount of complexometically neutral magnesium salt of EDTA is added to the buffer. The sharpness of the end point increases with increasing pH. However, the specified pH of 10.0 ± 0.1 is a satisfactory compromise. At a higher pH i.e. at about 12.0 Mg^{++} ions precipitate and only Ca^{++} ions remain in solution. At this pH murexide (ammonium purpurate) indicator forms a pink colour with Ca^{++}. When EDTA is added Ca^{++} gets complexed resulting in a change from pink to purple which indicates end point of the reaction. To minimise the tendency towards $CaCO_3$ precipitation limit the duration of titration period to 5 minutes.

9.2 Apparatus

a. Conical flasks 100mL
b. Burette
c. Pipette
d. Spatula

9.3 Reagents and standards

a. Buffer solution: Dissolve 16.9 g NH_4Cl in 143mL NH_4OH. Add 1.25 g magnesium salt of EDTA to obtain sharp change in colour of indicator and dilute to 250mL. If magnesium salt of EDTA (AR grade) and 780 mg $MgSO_4.7H_2O$ or 644 mg $MgCl_2.6H_2O$ in 50mL distilled water. Add this to above solution of NH_4Cl in NH_4OH and dilute to 250mL.

b. Inhibitor: Dissolve 4.5g hydroxylamine hydrochloride in 100mL 95% ethyl alcohol or isopropyl alcohol.

   or

   Add 250mg NaCN in powder form to a sample adjusted to a pH 6.0 or above. Add sufficient buffer to adjust to pH 10.0±0.1 (NaCN is extremely poisonous. Take extra precautions in it use).

   or

   Dissolve 5.0g sodium nonahydrate ($Na_2S.9H_2O$) or 3.7g $Na_2S.5H_2O$ in 100mL

distilled water. Exclude air with a tightly fitting rubber stopper. This inhibitor deteriorates through air oxidation.

or

MgCDTA: Add 250mg per 100mL sample magnesium salt of 1,2-cyclohexane-diamine tetra acetic acid and dissolve completely before adding buffer solution. Use this complexing agent to avoid using toxic or odorous inhibitors when interfering substances are present in concentration that affect end point but will not contribute significantly to hardness value.

c. Eriochrome black T indicator: Mix 0.5g dye with 100g NaCl to prepare dry powder.

d. Murexide indicator: Prepare a ground mixture of 200mg of murexide with 100g of solid NaCl.

e. Sodium hydroxide 2N: Dissolve 80g NaOH and dilute to 1000mL.

f. Standard EDTA solution 0.01 M: Dissolve 3.723 g EDTA sodium salt and dilute to 100mL. Standardise against standard Calcium solution 1mL = 1 mg $CaCO_3$.

g. Standard calcium solution: Weigh accurately 1g $CaCO_3$ (AR grade) and transfer to 250mL conical flask. Place funnel in the neck of a flask and add 1+1 HCl till $CaCO_3$ dissolves completely. Add 200mL distilled water and boil for 20-30 minutes to expel CO_2. Cool and add few drops of methyl red indictor. Add 8N NH_4OH drop-wise till intermediate orange colour develops. Dilute to 1000mL to obtain 1mL = 1mg $CaCO_3$

9.4 Sample collection, preservation and storage

The procedure is given in detail in collection preservation and storage of the sample.

9.5 Calibration

The EDTA solution needs be standardize against standard calcium solution such that the strength of EDTA will be 1mL = 1mg as $CaCO_3$

9.6 Procedure

9.6.1 Total hardness

a. Take 25 or 50mL well mixed sample in porcelain dish or conical flask.

b. Add 1-2mL buffer solution followed by 1mL inhibitor.

c. Add a pinch of Eriochrome black T and titrate with standard EDTA (0.01M) till wine red colour changes to blue, note down the volume of EDTA required (A).

d. Run a reagent blank. Note the volume of EDTA (B).

e. Calculate volume of EDTA required by sample, C = (A-B).

f. For natural waters of low hardness, take a larger sample volume, i.e. 100-1000mL for titration and add proportionately larger amounts of buffer, inhibitor and indicator. Add standard EDTA titrant slowly from a micro burette and run a blank using redistilled, deionised water of the same volume as sample. Apply blank correction for computing the results.

9.6.2 Calcium hardness

a. Take 25 or 50mL sample in a porcelain dish.

b. Add 1mL NaOH to raise pH to 12.0 and a pinch of Murexide indicator.

c. Titrate immediately with EDTA till pink colour changes to purple.

   Note the volume of EDTA required (A^1).

d. Run a reagent blank. Note the mL of EDTA required (B^1) and keep it aside to compare end points of sample titrations.

e. Calculate the volume of EDTA required by sample, $C^1 = A^1 - B^1$.

f. Standardise the EDTA (0.1M) solution following the procedure of calcium hardness from 1 to 4, using standard calcium solution.

Titrations are best conducted at or near normal room temperatures. The colour change becomes impractically slow as the sample approaches freezing temperature. Indicator decomposition presents a problem in hot water.

The pH specified in the recommended procedure may result in $CaCO_3$
Titrations are best conducted at or near normal room temperatures. The colour change becomes impractically slow as the sample approaches freezing temperature. Indicator decomposition presents a problem in hot water.

The pH specified in the recommended procedure may result in $CaCO_3$ precipitation. Although the titrant can redissolve such precipitates slowly, a drafting end point often will yield low results. A time of 5 min of the overall procedure minimises the tendency for to $CaCO_3$ precipitate.

Dilute sample with distilled water to reduce $CaCO_3$ concentration. If precipitation occurs at the dilution of 1+1, use following modifications because too small a volume contributes a systematic error due to the burette-reading error.

If the approximate hardness is known or is determined by a preliminary titration, add 90% or more titrant to sample before adjusting pH with buffer.

9.7 Calculation

a. Total hardness as $CaCO_3$ mg/L = C x D x 1000 / mL sample

where, C = volume of EDTA required by sample
D = mg $CaCO_3$ equivalent to 1mL EDTA titrant

b. Calcium hardness $CaCO_3$ as mg/L = C1 x D x 1000 / mL sample
where C' = volume of EDTA used by sample
D = mg $CaCO_3$ equivalent to 1mL EDTA titrant

c. Magnesium hardness = Total hardness as $CaCO_3$, mg/L - Calcium hardness as $CaCO_3$, mg/L

d. Alkaline (Carbonate) hardness and non-alkaline (non-carbonate) hardness

These types of hardness can be calculated from total hardness and total alkalinity as follows:

i. If total hardness as $CaCO_3$ > total alkalinity as $CaCO_3$

Then, a. Alkaline hardness = Total alkalinity

b. Non-alkaline hardness = Total hardness Total alkalinity

ii If total hardness as $CaCO_3$ < total alkalinity as $CaCO_3$

Then, a. Alkaline hardness = Total hardness

b. Nonalkaline hardness = Nil

9.8 Precision and Bias

Run a blank to check the analyte contamination. Analyse the sample in duplicate to see the precision of method. A synthetic sample containing 610 mg/L total hardness, contributed by 108 mg/L calculate and 82 mg/L showed 2.9% standard deviation and 0.8% relative error, when analysed by 56 different laboratories.

9.9 Interferences

Some metal ions interfere by causing fading or indistinct end points or by stoichiometric of EDTA but can be reduced by addition of inhibitors. Suspended or colloidal organic matter may also interfere with the end point. This interference can be eliminated by evaporating 50mL sample to dryness on a steam bath and then heating in a muffle furnace at 550°C. Residue may be dissolved in 20mL of 1N hydrochloric acid and on neutralization to pH 7 with 1N sodium hydroxide, volume be made to 50mL with distilled water. Run a reagent blank following the same procedure.

9.10 Pollution prevention and waste management

Refer section 7.5.

9.11 References

1. Goetz, C.A. and R.C. Smith, (1959). Evaluation of various methods and reagents for total hardness and calcium hardness in water. lowa State J.Sci. 34:81.
2. Schwarzenbach, G.and H. Flaschika, (1969). Complexometric Titrations, 2nd Ed., Barnes and Noble, Inc., New York, N.Y.
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10

Biochemical Oxygen Demand (BOD)

Introduction:

The Biochemical Oxygen Demand (BOD) is an empirical standardized laboratory test which measures oxygen requirement for aerobic oxidation of decomposable organic matter and certain inorganic materials in water, polluted waters and wastewater under controlled conditions of temperature and incubation period. The quantity of oxygen required for above oxidation processes is a measure of the test. The test is applied for fresh water sources (rivers, lakes), wastewater (domestic, industrial), polluted receiving water bodies, marine water (estuaries, coastal water) and also for finding out the level of pollution, assimilative capacity of water body and also performance of waste treatment plants. Guideline BOD values for classification of raw untreated water is given below (Table 10.1: I).

Table 10.1: I: Guideline BOD values for classification of raw untreated water

Quality class	Designated best use	BOD value	Note
A	Drinking water source without conventional treatment but with chlorination	2 or less	Could cause problems in treatment, larger CL_2 demand and residual taste/odour problem.
B	Drinking water source with conventional treatment	3 or less	

Source: CPCB, July 2001.

A. Titrimetric method

10.1 Principle

This test measures the oxygen utilised for the biochemical degradation of organic material (carbonaceous demand) and oxidation of inorganic material such as sulphides and ferrous ions during a specified incubation period. It also measures the oxygen used to oxidize reduced forms of nitrogen (nitrogenous demand) unless their oxidation is prevented by an inhibitor. Temperature effects are held constant by performing a test at fixed temperature.

The methodology of BOD test is to compute a difference between initial and final DO of the samples incubation. Minimum 1.5 L of sample is required for the test. DO is estimate by iodometric titration.

Since the test is mainly a bio-assay procedure, it is necessary to provide standard conditions of temperature, nutrient supply, pH (6.5-7.5), adequate population of microorganisms and absence of microbial-growth-inhibiting substances. The low solubility of oxygen in water necessitates strong wastes to be diluted to ensure that the demand does not increase the available oxygen. A mixed group of microorganisms should be present in the sample; otherwise, the sample has to be seeded. Generally, temperature is controlled at 20°C and the test is conducted for 5 days, as 70 to 80% of the carbonaceous wastes are oxidized during this period. The test can be performed at any other temperature provided the correlation between BOD_5 20°C is established under same experimental condition (for example BOD_3, 27°C) is equivalent to BOD_3, 27°C) for Indian conditions. While reporting the results, the incubation period in days and temperature in °C is essential to be mentioned.

10.2 Equipment and apparatus

a. BOD bottles 300mL capacity (clean with a detergent, rinse thoroughly and drain before use) with a water seal.

b. Incubator or water-bath to be controlled at 20°C or at any desired temperature 1°C. Exclude all light to prevent photosynthetic production of DO.

10.3 Reagents and standards

All reagents listed in DO estimation are used for BOD. In addition following reagents are required:

a. Phosphate buffer: Dissolve 8.5g KH_2PO_4, 21.75g K_2HPO_4, 33.5g $Na_2HPO_4.7H_2O$ and 1.7g NH_4C; in distilled water and dilute to 1000mL. The pH should be 7.2without further adjustment. Discard reagent if there is any sign of biological growth.

b. Magnesium sulphate: Dissolve 22.5g $MgSO_4.7H_2O$ in about 700mL of distilled water and dilute to 1 Litre.

c. Calcium chloride: Dissolve 27.5g anhydrous $CaCl_2$ in about 7000mL of distilled water and dilute to 1 Litre.

d. Ferric chloride: Dissolve 0.25g $FeCl_3.6H_2O$ in about 700mL of distilled water and dilute to 1 L.

e. Sodium sulphate solution 0.025N: Dissolve 1.575g Na_2SO_3 in distilled water and dilute to 1000mL. Solution should be prepared daily.

f. Acid and Alkali solutions 1N: Prepare 1N H_2SO_4 and 1N NaOH or neutralization of caustic or acidic samples.

g. Nitrification inhibitor: 2-chloro-6-(trochloromethyl) pyridine [Nitrification inhibitor 2570-24 (2.2% TCMP), Hach Co. equivalent]

h. Glucose-glutamic acid solution: Dry reagent grade glucose and glutamic acid at 103°C for 1h. Dissolve 150 mg glucose and 150mg glucose acid in distilled water and dilute to 1000mL. Prepare fresh immediately before use.

10.4 Sample collection, preservation and storage

Grab or composite samples are collected. Keep composite samples at or below 4°C during compositing. Samples for BOD may degrade significantly during storage. Minimise reduction of BOD by analyzing samples promptly or by cooling it to near freezing temperature during storage. The maximum holding time recommended between collection and analysis is 48 hours. Warm chilled samples to 20-27°C ± 3°C before analysis. State storage time and condition as part of results.

10.5 Procedure

Preparation of dilution water:

a. The source of dilution water may be distilled water, tap or receiving-stream water free of biodegradable organics and bioinhibitory substances such as chlorine or heavy metals.

b. Aerate the required volume of dilution water in a suitable bottle by bubbling clean-filtered compressed air for sufficient time to attain DO saturation at room temperature or at 20°C/27°C. Before use stabilise the water at 20°C/27°C.

c. Add 1mL each of phosphate buffer, magnesium sulphate, calcium chloride and ferric chloride solutions in that order for each Litre of dilution water. Mix well. Quality of dilution water may be checked by incubating a BOD bottle full of dilution water for 5 days at 20°C for 3 days at 27°C. DO uptake of dilution water should not be more than 0.2mg/L and preferable not more than 0.1mg/L.

d. For wastes which are not expected to have sufficient microbial population, seed is essential. Preferred seed is effluent from a biological treatment system. Where this is not available, supernatant from domestic wastewater (domestic sewage) settled at room temperature for at least 1h but not longer than 36hours is considered sufficient in the proportion 1-2mL/L of dilution water. Adopted microbial population can be obtained from the receiving water microbial population can be obtained from the receiving water body preferably 3-8 km below the point of discharge. In the absence of such situation, develop an adapted seed in the laboratory.

e. Determine BOD of the seeding material. This is seed control. From the value of seed control determine seed DO uptake. The DO uptake of seeded dilution water should be between 0.6mg/L and 1mg/L.

Sample preparation:

a. Neutralise the sample to pH 7, if it is highly acidic or alkaline.

b. The sample should be free from residual chlorine. If it contains residual chlorine remove it by using $Na_2S_2O_3$ solution as described below.

c. Take 50mL of the sample and acidify with addition of 10mL 1 + 1 acetic acid. Add about 1g Kl. Titrate with 0.025N $Na_2S_2O_3$, using starch indicator. Calculate the volume of $Na_2S_2O_3$ required per Litre of the sample and accordingly add to the sample to be tested for BOD.

d. Certain industrial wastes contain toxic metals, e.g. planting wastes. Such samples often require special study and treatment.

e. Bring samples to 20 ± 1°C before making dilutions.

f. If nitrification inhibition is desired, add 3mg 2-chloro-6-(trichloromethyl) pyridine (TCMP) to each 300mL bottle before capping or add sufficient amount to the dilution water to make a final concentration of 30mg/L. Note the use of nitrogen inhibition in reporting results.

g. Samples having high DO contents, DO ≥ 9mg/L should be treated to reduce the DO content to saturation at 20°C. Agitate or aerate with clean, filtered compressed air.

Dilution of sample: Dilutions that result in a residual DO of at least 1mg/L and DO uptake of at least 2mg/L produce reliable results. Make several dilutions of the pre-treated sample so as to obtain about 50% depletion of DO or DO uptake of 2mg/L. Prepare dilutions as follows:

Siphon out half the required volume of seeded dilution water in a graduated cylinder or volumetric flask without entraining air. Add the desired quantity of mixed sample and dilute to the appropriate volume by siphoning dilution water. Mix well with plunger type mixing rod to avoid entraining air.

General guidelines for dilution range are as follows:

0.1% to 1%	:Strong trade waste
1% to 5%	:Raw or settled sewage
5% to 25%	:Treated effluent
25% to 100%	:River water

Sample processing:

a. Siphon the diluted or undiluted sample in three labeled bottles and stopper immediately.

b. Keep 1 bottle for determination of the initial DO and incubate 2 bottles at 20°C for 3days. See that the bottles have a water seal.

c. Prepare a blank in triplicate by siphoning plain dilution water (without seed) to measure the O_2 consumption in dilution water.

d. Also prepare a seed blank in triplicate to measures BOD of seed for correction of actual BOD.

e. Determine DO in a BOD test can in the blank on initial day and end of incubation period by Winkler method as described for DO measurement.

f. DO estimation in a BOD test can also be done by membrane electrodes. A DO probe with a stirrer is used to determine initial and final DO after incubation in BOD samples. The semi-permeable membrane provided in the DO probe acts as a diffusion barrier against impurities between sensing element and sample.

10.6 Calculations

Calculate BOD of the sample as follows:

a. When dilution water is not seeded

$$\text{BOD as } O_2 \text{ mg/L} = \frac{(D_1 - D_2) \times 100}{\% \text{ dilution}}$$

b. When dilution is seeded

$$\text{BOD } O_2 \text{ mg/L} = \frac{(D_1 - D_2) - (B_1 - B_2) \times 100}{\% \text{ dilution}}$$

c. When material is added to sample or to seed control

$$\text{BOD } O_2 \text{ mg/L} = \frac{(D_1 - D_2) - (B'_1 \times B'_2) \times F \times 100}{\% \text{ dilution}}$$

where,

D_1 = DO of sample immediately after preparation, mg/L

D_2 = DO of sample after incubation period, mg/L

B_1 = DO of blank (seeded dilution water) before incubation, mg/L

B_2 = DO of blank (seeded dilution water) after incubation, mg/L

F = ration of seed in diluted sample to seed in seed control (Vol. Of seed in diluted sample / Vol. of seed in seed control)

B_1' = DO of seed control before incubation, mg/L

B_2' = DO of seed control after incubation, mg/L

In calculations, do not make corrections for DO uptake in dilution water.

10.7 Precision and Bias

For reliable results following conditions are essential:

a. Minimum depletion of DO of 2mg/L
b. Minimum residual DO of 1mg/L at the end of test period
c. 5days 20°C or 3days 27°C BOD value of 5mg/L can be measured directly without dilution
d. For reproducible and accurate results, perform the test in duplicate
e. Check the quality of reagents and dilution water
f. Perform glucose-glutamic acid check to get the value of BOD for known synthetic chemical.

Minimum detection limits is 1mg/L. Because BOD test is bioassay, the results are influenced by many factors, viz. operating conditions like pH, nutrients, buffers, toxicants, seed material, etc. The standard check with the Glucose-glutamic acid (GG) is intended for evaluating reliability of analytical technique adopted. For 300mg/L mixed primary standard of GG, average BOD would be 198 mg/L with a standard deviation of 30.5 mg/L. There is no measurement of establishing bias of the BOD procedure.

10.8 Interference

Since DO estimation is the basis of BOD test, sources of interference in BOD test are the same as in the DO test. In addition, lack of nutrients in dilution water, lack of an acclimated seed organisms and presence of heavy metals or other toxic materials such as residual chlorine are other sources of interferences in this test. Neutralise the sample to pH to 7.5 using pH meter. Remove high residual chlorine by adding predetermined sodium sulphate solution.

10.9 Pollution prevention and waste management

Once the expertise is developed, use 125mL DO bottles instead of 300mL for volume reduction. The acidic liquid samples after titration of DO should be flushed with sufficient water for adequate dilution.

B. Respirometric method

10.10 Principle

The respirometric method provides direct measurement of O_2 consumed by microorganisms from an air in a closed vessel under conditions of constant temperature and agitation. The procedure prescribed by the manufacturer should be adopted. This method measures O_2 uptake continuously over a specified time. Commercially, various types of respirometers are available. Most respirometers permit data collection and processing. Reaction vessel contents are mixed by using a magnetic or mechanical stirring device. All respirometers remove carbon dioxide produced during biological growth by adsorbent within closed reaction chamber.

The measuring principle of "Oxitop" control measuring system is as follows:

This instrument falls under respirometric measurement of BOD. The sample bottles are filled according to the "standard data" suggested by manufacturer for BOD measuring range with assigned sample volume. The micro-organisms draw oxygen to degrade organic substances from the amount of air remaining in the partially filled closed system. The carbon dioxide formed by this absorbed by NaOH. Due to the reduction in the amount of oxygen, the presence in the bottle sinks. This change is detected and stored by the measuring head. After the data transfer to the controller, it is used to determine the BOD value. The instruction provided by supplier should by strictly followed. Comparative performance of conventional titrimetric method and respirometric method should be carried out for precision and reliability of the test before adopting for routine analysis.

10.11 Precision and Bias

Precision and Bias: Refer section 10.7.

10.12 Pollution prevention and waste management

Refer section 10.9.

10.13 Bibliography

1. Standard Methods for the Examination of Water and Wastewater. APHA, AWWA and WEF, 21st Edition, 2005.
2. Sawyer Clair N., Perry Z. McCarty, Gener F. Parkin. Chemistry for Environmental Engineering, 4th Edition.
3. CPCB document stating the guidelines values for various water bodies. Rationalization and Optimisation of Water Quality Monitoring Network, CPCB, MINARS, IS-2001-2001.

11 ALKALINITY

11.1 Principle

Alkalinity of sample can be estimated by titrating with standard sulphuric acid (0.02N) at room temperature using phenolphthalein and methyl orange indicator. Titration to decolourisation of phenolphthalein indicator will indicate complete neutralization of OH^- and ½ of $CO3^-$, while sharp change from yellow to orange of methyl orange indicator will indicate total alkalinity (complete neutralisation of OH^-, CO_3^-, HCO_3^-).

11.2 Apparatus

a. Beakers: The size and form will depend upon the electrode and the size of the sample to be used for determination of alkalinity.

b. Pipettes (volumetric)

c. Flasks (volumetric): 1000mL, 200mL, 100mL

11.3 Reagents and standards

a. Standard H_2SO_4, 0.02 N: Prepare 0.1N H_2SO_4 by diluting 3mL conc. H_2SO_4 to 1000mL. Standardise it against standard 0.1N Na_2CO_3 solution. Dilute appropriate volume of H2SO4 to 1000mL to obtain standard 0.02 H_2SO_4.

b. Phenolphthalein indicator: Dissolved 0.5g in 500mL 95% ethyl alcohol. Add 500mL distilled water. Add dropwise 0.02N NaOH till faint pink colour appears (pH 8.3).

c. Methyl orange indicator: Dissolve 0.5g and dilute to 1000mL with CO_2 free distilled water (pH 4.3-4.5).

   OR

   Bromo-cresol green indicator: Dissolve 0.1g bramocresol green, sodium salt, in 100mL distilled water (pH 4.5).

11.4 Calibration

Standardise the pH meter by using pH buffers. Follow the instructions given in the manual of pH meter.

11.5 Procedure

a. Take 25 or 50mL sample in a conical flask and add 2-3 drops of phenolphthalein indicator.

b. If pink colour develops titrate with 0.02N H_2SO_4 till disappears or pH is 8.3. Note the volume of H_2SO_4 required.

c. Add 2-3 drops of methyl orange to the same flask, and continue titration till yellow colour changes to orange. Note the volumes of H_2SO_4 required.

d. In case pink colour does not appear after addition of phenolphthalein continue as above.

e. Alternatively, perform potentiometric titration to preselected pH using appropriate volume of sample and titration assembly. Titrate to the end point pH without recording intermediate pH. As the end point is approached make smaller additions of acid and be sure that pH equilibrium is reached before adding more titrant. The following pH values are suggested as equivalence points for corresponding alkalinity as mg $CaCO_3$/L (Table 11.5: I).

Table 11.5: I: End point pH values

Alkalinity range and Nature of sample	End point pH	
	Total Alkalinity	Phenolphthalein Alkalinity
Alkalinity, mg $CaCO_3$/L:		
30	4.9	8.3
150	4.6	8.3
500	4.3	8.3
Silicates, phosphates known or suspended	4.5	8.3
Industrial waste or complex system	4.5	8.3
Routine or automated analyses	4.5	8.3

11.6 Calculations

Calculate total (T), phenolphthalein (P) alkalinity as follows:

P-alkalinity, as mg $CaCO_3$/L = A x 1000/mL sample
T-alkalinity, as mg $CaCO_3$/L = B x 1000/mL sample

In case H_2SO_4 is not 0.02 N apply the following formula:

Alkalinity, as mg $CaCO_3$/L = A/B x N x 50000 / mL of sample

Where,
A = mL of H_2SO_4 required to bring the pH to 8.3
B = mL of H_2SO_4 required to bring the pH to 4.5
N = normality of H_2SO_4

Once, the phenolphthalein and total alkalinities are determined, three types of alkalinities, i.e. hydroxide, carbonate and bicarbonate are easily calculated from the table given as under:

Table 11.6: I: Type of alkalinity

Values of P and T	Type of Alkalinity		
	OH^-	CO_3^{--}	HCO_3^-
P = O	0	0	T
P<1/2T	0	2P	T-2P
P = 1/2T	0	2P	0
P>1/2T	2P-T	2(T-P)	0
P = T	T	0	0

Once carbonate and bicarbonate alkalinities are known, then their conversions to milligrams CO_3^{--} or HCO_3^-/L are possible.

mg CO_3^{--}/L = Carbonate alkalinity mg $CaCO_3$/L x 0.6

mg HCO_3 = Bicarbonate alkalinity mg $CaCO_3$/L x 1.22

from above, molar concentration may be obtained as follows:

$[CO_3^{--}]$ = mg/L CO_3 / 60000

$[HCO_3^-]$ = mg/L HCO_3^- / 61000

Conductivity

12

Introduction:

Conductivity is the capacity of water to carry an electrical current and varies both with number and types of ions in the solutions, which in turn is related to the concentration of ionized substances in the water. Most dissolved inorganic substances in water are in the ionized form and hence contribute to conductance.

A. Instrumental Method

12.1 Principle

This method is used to measure the conductance generated by various ions in the solution/water. Rough estimation of dissolved ionic contents of water sample can be made by multiplying specific conductance (in mS/cm) by an empirical factor which may vary from 0.55 to 0.90 depending on the soluble components of water and on the temperature of measurement.

Conductivity measurement gives rapid and practical estimate of the variations in the dissolved mineral contents of a water body.

12.2 Apparatus and equipment

a. Self-contained conductance instruments: (Conductivity meter). These are commercially available.

b. Thermometer, capable of being read to the nearest 0.1°C and covering the range 10-50°C.

c. Conductivity Cells: The cell choice will depend on the expected range of conductivity and the resistance range of the instrument. Experimentally check the range of the instruments assembly by comparing the instrumental results with the true conductance of the potassium chloride solution.

12.3 Reagents and standards

Conductivity Water: The conductivity of the water should be less than 1 $_{m}$mho/cm; Standard

potassium chloride: 0.01M; dissolve 745.6mg anhydrous KCl in conductivity water and make up to 1,000mL at 25°C. This is the standard reference solution, which at 25°C has a specific conductance of 1,413 $_{m}$mhos/cm. It is satisfactory for most waters when using a cell with a constant between 1 and 2. Store the solutions in glass stoppered Pyrex bottles.

12.4 Procedure

Conductivity can be measured as per the instruction manual supplied with the instrument and the results may be expressed as mS/m or $_{m}$S/cm. Note the temperature at which measurement is made. Conductivity meter needs very little maintenance and gives accurate results. However few important points in this respect are:

a. Adherent coating formation of the sample substances on the electrodes should be avoided which requires thorough washing of cell with distilled water at the end of each measurement.

b. Keep the electrode immersed in distilled water

c. Organic material coating can be removed with alcohol or acetone followed by washing with distilled water.

12.5 Calculation

Follow the instruction manual.

12.6 Precision and Bias

Three synthetic unknown samples were tested with the results as shown in Table 12.6: I. After every 10 samples check the meter by using standard potassium chloride solution (0.01 M) which has conductivity 1413 $_{m}$mho/cm.

Table 12.6: I: Method performance in conductivity measurements

Conductivity $_{m}$mhos/cm	No. of Results	Relative Standard	Relative Error Deviation
147.0	117	8.6	9.4
3.3.0	120	7.8	1.9
228.0	120	8.4	3.0

12.7 Interferences

Conductivity measurement is affected by:

a. The nature of the various ions, their relative concentration and the ionic strength of water.

b. Dissolved CO_2

c. Turbidity.

d. Temperature (For precise work, the conductivity must be determined at 25°C.

12.8 References

1. Jones, G. and B. C. Bradshaw, (1933). The measurement of the conductance of electrolytes: A redetermination of the conductance of the standard potassium chloride solutions in absolute units, J. Amer, Chem. Soc. 55:1780.

2. Standard Methods for the Examination of Water and Wastewater, APHA, AWWA and WEF, 21st Edition, 2005.

13 Chlorine (Residual)

Introduction:

The chlorination of water supplies and polluted waters serves primarily to destroy or deactivate disease-producing micro-organisms. A secondary benefit, particularly in treating drinking water, is the overall improvement in water quality resulting from the reaction of chlorine with ammonia, iron, manganese, Sulphide and some organic substances.

Chlorination may produce adverse effects. Taste and odour characteristics of phenols and other organic compounds present in a water supply may be intensified. Potentially carcinogenic chloro-organic compounds such as chloroform may be formed. Combined chlorine formed on chlorination of ammonia-or amine-bearing waters adversely affects some aquatic life. To fulfil the primary purpose of chlorination and to minimise any adverse effects, it is essential that proper testing procedures be used with a foreknowledge of the limitations of the analytical determination.

Chlorine applied to water in its molecular or hypochlorite form initially undergoes hydrolysis to form free chlorine consisting of aqueous molecular chlorine, hypochlorous acid and hypochlorite ion. The relative proportion of these free chlorine forms is pH and temperature-dependent. At the pH of most waters, hypochlorous acid and hypochlorite ion will predominate.

Free chlorine reacts readily with ammonia and certain nitrogenous compounds to form combined chlorine. With ammonia, chlorine reacts to form the chloramines: monochloramine, dichloramine and nitrogen trichloride. The presence and concentrations of these combined forms depend chiefly on pH, temperature, initial chlorine-to-nitrogen ratio, absolute chlorine demand and reaction time. Both free and combined chlorine may be present simultaneously. Combined chlorine in water supplies may be formed in the treatment of raw waters containing ammonia or by the addition of ammonia or ammonium salts. Chlorinated wastewater effluents, as well as certain chlorinated industrial effluents, normally contain only combined chlorine. Historically the principal analytical problem has been to distinguish between free and combined forms of chlorine.

13.1 Principle

Chlorine will liberate free iodine from potassium iodine (KI) solutions at pH 8 or less. The liberated iodine is titrated with a standard solution of sodium thiosulphate ($Na_2S_2O_3$) with

starch as the indicator. The liberated iodine is directly proportional to the concentration of chlorine present in sample. Titrate at pH 3 to 4 because the reaction is not stoichiometric at neutral pH due to partial oxidation of thiosulphate to sulphate.

Select a sample volume that will require not more than 20mL 0.01N sodium thiosulphate. For residual chlorine concentration of 1 mg/L or less, 100mL sample for chlorine range 1-10 mg/L, 500mL for chlorine above 10mg/L and proportionally less as per chlorine concentration.

13.2 Analytical methods

A. Iodometric method

The iodometric method is suitable for measuring total chlorine concentrations greater than 1 mg/L. All acidic iodometric methods suffer from interferences, generally in proportion to the quantity of potassium iodine (KI) and H^+ added.

13.3 Reagents and standards

a. Acetic acid, conc. (glacial)

b. Potassium iodide, KI, crystals

c. Standard sodium thiosulphate, 0.1N: Dissolve 25g $Na_2S_2O_3.5H_2O$ in 1L freshly boiled distilled water and standardise against Potassium bi-iodate or potassium dichromate after at least 2 weeks storage. This initial storage is necessary to allow oxidation of any sulphate ion present. Use boiled distilled water and add a few mLs chloroform ($CHCl_3$) to minimise bacterial decomposition.

d. Starch indicator solution: To 5g Starch (potato, arrowroot, or soluble), add a little cold water and grind in a mortar to a thin paste. Pour into 1L of boiling distilled water, stir, and let settle overnight. Use clear supernate. Preserve with 1.25g salicylic acid, 4g zinc chloride, or a combination of 4g sodium proportionate and 2g sodium azide/L starch solution. Some commercial starch substitutes are satisfactory.

e. Standard iodine, 0.1N: Dissolve 40g KI in 25mL distilled water. Add 13g resublimed iodine and stir until dissolved. Transfer to a 1L volumetric flask and dilute to make volume up to mark.

f. Dilute standard iodine, 0.0282N: Dissolve 25g KI in a little distilled water in a volumetric flask, and add to correct amount of 0.1N iodine solution to get 0.0282N solution; standardise this solution daily.

13.4 Sample collection, preservation and storage

Chlorine in aqueous solution is not stable, and the chlorine content of samples or solutions, particularly weak solutions, will decrease rapidly. Exposure to sunlight or other strong light or agitation will accelerate the reduction of chlorine. Therefore, chlorine determination

should be done immediately after sampling, avoiding excessive light and agitation. The sample should not be stored for analyses of residual chlorine.

13.5 Calibration

Standardise 0.1N $Na_2S_2O_3$ by one of the following.

a. Iodate method Dissolve 3.249g anhydrous potassium bi-iodate. $KH(IO_3)_2$, primary standard quality; or 3.567g KIO_3 dried at 103 ± 2°C for 1h, in distilled water and dilute to 1000mL to yield a 0.1N solution. Store in a glass-stoppered bottle.

b. To 80mL distilled water, add, with constant stirring, 1mL conc. H_2SO_4, 10.0mL 0.1N $KH(IO_3)_2$, and 1g KI. Titrate immediately with 0.1N $Na_2S_2O_3$ titrant until the yellow colour of the liberated iodine almost is discharged. Add 1mL starch indicator solution and continue titrating until the blue colour disappears.

c. Dichromate method Dissolve 4.904g anhydrous potassium dichromate, $K_2Cr_2O_7$, of primary standard quality, in distilled water and dilute to 1000mL to yield a 0.1N solution. Store in a glass-stoppered bottle.

d. Proceed as in the iodate method with the following exceptions: substitute 10.00mL 0.1N $K_2Cr_2O_7$ for iodate and let reaction mixture stand 6min in the dark before titrating with 0.1N $Na_2S_2O_3$ titrant.

   Normality $Na_2S_2O_3$ = 1 / mL $Na_2S_2O_3$ consumed

e. Standard sodium thiosulfate titrant, 0.01N or 0.025N: Improve the stability of 0.01N or 0.025N by $Na_2S_2O_3$ diluting an aged 0.1N solution, made as directed above, with freshly boiled distilled water. Add 4g sodium borate and 10mg mercuric iodide/L solution. For accurate work, standardise this solution daily in accordance with the directions given above, using 0.01N or 0.025N iodate or $K_2Cr_2O_7$. Use sufficient volumes of these standard solutions so that their final dilution is not greater than 1+4. To speed up operations where many samples are to be titrated, use an automatic burette of a type in which rubber does not come in contact with the solution. Standard titrants, 0.01N and 0.025N, are equivalent, respectively to 354.5 µg and 886.3 µg Cl as Cl_2/mL.

13.6 Procedure

a. Volume of sample: Select volume that will require not more than 20mL 0.01N $Na_2S_2O_3$ and not less than 0.2mL for the starch-iodide end point. For a chlorine range of 1 to 10mg/L., use a 500mL sample: above 10mg/L, use proportionately less sample. Use smaller samples and volumes of titrant with the amperometric end point.

b. Preparation for titration: Place 5ml acetic acid, or enough to reduce the pH between 3.0 and 4.0, in a flask or white porcelain casserole. Add about 1g KI estimated on a spatula. Pour sample in and mix with a stirring rod.

c. Titration: Titrate away from direct sunlight. Add 0.025N or 0.01N $Na_2S_2O_3$ from a

burette until the yellow colour of the liberated iodine almost id discharged. Add 1mL starch solution and titrate $Na_2S_2O_3$ instead of 0.01N, then, with a 1L sample, 1drop is equivalent to about 50g/L. It is not possible to discern the end point with greater accuracy.

13.7 Calculation

For standardizing chlorine solution for temporary standards:

mg Cl as Cl_2/mL = (A ± B) x N x 35.45 / mL sample

For determining total available residual chlorine in a water sample:

mg Cl as Cl_2/mL = (A ± B) x N x 35450 / mL sample

where: A = mL titration for sample.
B = mL titration for blank (positive or negative), and
N = normality of $Na_2S_2O_3$

13.8 Precision and Bias

Correct result of sample titration by determining blank contributed by oxidising or reducing agent impurities. The blank also compensates for the concentration of iodine bound to starch at the end point.

Take a volume of distilled water corresponding to the sample used for titration, Add 5mL acetic acid, 1g Kl, and 1mL starch solution. Perform blank titration as given in a and b below, whichever applies.

a. If a blue colour develops, titrate with 0.01N or 0.025N $Na_2S_2O_3$ to disappearance of blue colour and record result. B is negative.

b. If no blue colour occurs, titrate with 0.0282N iodine solution until a blue colour appears. Back-titrate with 0.01N 0.025N $Na_2S_2O_3$ and record the difference. B is positive.

   Before calculating the chlorine concentration, subtract the blank titration of a from the sample titration; or, if necessary, add the net equivalent value of the blank titration of B.

c. Analyse the sample in duplicate.

13.9 Interferences

Oxidised forms of manganese and other oxidising agents interfere. Reducing agents such as organic sulphides also interfere. Although the neutral titration minimises the interfering

effect of ferric and nitrite ions, the acid titration is preferred because some forms of combined chlorine do not react at pH 7. Use only acetic acid for the acid titration; sulphuric acid (H_2SO_4) will increase interferences; never use hydrochloric acid (HCl).

Minimum detectable concentration: The minimum detectable concentration approximates 40g Cl as Cl_2/L if 0.01N $Na_2S_2O_3$ is used with a 1000mL sample. Concentrations below 1mg/L cannot be determined accurately by the starch-iodine end point in this method.

13.10 Pollution prevention and waste management

The chemicals and reagent are used in very dilute form. During washing further dilution will take place. It may not pose any problem of disposal.

Chemical Oxygen Demand (COD)

14

Introduction:

Chemical Oxygen Demand (COD) test determines the oxygen requirement equivalent of organic matter that is susceptible to oxidation with the help of a strong chemical oxidant. It is an important, rapidly measured parameters as a means of measuring organic strength for streams and polluted water bodies. The test can be related empirically to BOD, organic carbon or organic matter in samples from a specific source taking into account its limitations. The test is useful in studying performance evaluation of wastewater treatment plants and monitoring relatively polluted water bodies. COD determination has advantage over BOD determination. COD results can be obtained in 3-4 hrs as compared to 3-5days required for BOD test. Further, the test is relatively easy, precise, and is unaffected by interferences as in the BOD test. The intrinsic limitation of the test lies in its inability to differentiate between the biologically oxidisable and biologically inert material and to find out the system rate constant of aerobic biological stabilization.

A. Open Reflux method

14.1 Principle

The open reflux method is suitable for a wide range of wastes with a large sample size. The dichromate reflux method is preferred over procedures using other oxidants (e.g. 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 up to 95-100% of the theoretical value.

The organic matter gets oxidised completely by potassium dichromate ($K_2Cr_2O_7$) with silver sulphate as catalyst in the presence of concentrated H_2SO_4 to produce CO_2 and H_2O. The excess $K_2Cr_2O_7$ remaining after the reaction is titrated with ferrous ammonium sulphate [Fe $(NH_4)_2(SO_4)_2$]. The dichromate consumed gives the oxygen (O_2) required for oxidation of the organic matter. The chemical reactions involved in the method are as under:

a. $2K_2Cr_2O_7 + 8\,H_2SO_4 \rightarrow 2\,K_2SO_4 + 2Cr_2(SO_4)3 + 8\,H_2O + 3O_2$

b. $C_6H_{12}O_6 + 6O_2 \rightarrow 6CO_2 + 6H_2O$

c. $Cr_2O7^{--} + 6Fe^{++} + 14H^{+} \rightarrow 6Fe^{+++} + 2Cr^{3+} + 7H_2O$

14.2 Apparatus and equipment

a. 250 or 500mL Erlenmeyer flask with standard (24/40) tapered glass joints
b. Friedrich's reflux condenser (12 inch) with standard (24/40) tapered glass joints
c. Electric hot plate or six-unit heating shelf
d. Volumetric pipettes (10, 25, and 50mL capacity)
e. Burette, 50mL with 0.1mL accuracy
f. Burette stand and clamp
g. Analytical balance, accuracy 0.001g
h. Spatula
i. Volumetric flasks (1000mL capacity)
j. Boiling beads, glass
k. Magnetic stirrer and stirring bars.

14.3 Reagents and standards

a. Standard potassium dichromate solution, 0.25N (0.04167 M): Dissolve 12.259g $K_2Cr_2O_7$ dried at 103°C for 24h in distilled water and dilute to 1000mL. Add about 120mg sulphamic acid to take care of 6 mg/L NO_2 N.
b. Sulphuric acid reagent: Add 10g of Ag_2SO_4 to 1000mL concentrated H_2SO_4 and let stand for one to two days for complete dissolution.
c. Standard ferrous ammonium sulphate approx. 0.25N (0.25M): Dissolve 98g $Fe(NH_4)_2(SO_4)_2.6H_2O$ in about 400mL distilled water. Add 20mL concentrated H_2SO_4 and dilute to 1000mL.
d. Ferroin indicator: Dissolve 1.485g 1, 10-phenanthroline monohydrate and 695mg $FeSO_4.7H_2O$ in distilled water and dilute to 100mL.
e. Mercuric Sulphates: $HgSO_4$, crystals, analytical grade
f. Potassium hydrogen phthalate (KHP) Standard: Dissolve 425mg lightly crushed dried potassium hydrogen phthalate ($HOOC.C_6H_4.COOK$) in distilled water and dilute to 1000mL. This solution has a theoretical COD of 500µg O_2/mL. This solution is stable when refrigerated, up to 3 months in the absence of visible biological growth.

14.4 Sample collection, preservation and storage

Preferably collect samples in glass bottles. Homogenise samples containing settleable

solids. If there is delay in collection and analysis, preserve sample by acidification to pH≤2 using concentrated H_2SO_4. Samples can be preserved for maximum 7 days.

14.5 Calibration

Since the procedure involves chemical of organic matter by potassium dichromate as oxidizing agent, which is a primary standard, calibration is not applicable. For standardisation of ferrous ammonium sulphate, dilute 10mL standard $K_2Cr_2O_7$ to about 100mL. Add 10mL concentration of H_2SO_4 and allow it to cool. Titrate with ferrous ammonium sulphate (FAS) to be standardized using 2-3 drops of ferroin indicator. Calculate normally.

$$\text{Normality of FAS} = \frac{(\text{mL } K_2Cr_2O_7)\,(0.25)}{\text{mL FAS required}}$$

The deterioration of FAS can be decreased if it is stored in a dark bottle.

14.6 Procedure

Sample preparation: All samples high in solids should be blended for 2 minutes at high speed and stirred when an aliquot is taken for analysis. Select the appropriate volume of sample based on expected COD range, e.g. for COD range of 50-500 mg/L take 25-50mL of sample. Sample volume less than 25mL should not be pipetted directly, but serially diluted and then a portion of the diluted sample taken. Dilution factor should be incorporated in calculations.

a. 500mL of sample diluted to 1000mL = 0.5mL sample/mL of diluent, 50mL = 25mL of sample.

b. 100mL of sample diluted to 1,000mL = 0.1mL sample/mL diluent, 50mL of diluent = 5mL of sample.

Reflux of samples:

a. Place 0.4g $HgSO_4$ in a 250mL reflux sample

b. Add 20mL sample or an aliquot of sample diluted to 20mL with distilled water. Mix well.

c. Add clean pumic stones or glass beads.

d. Add 10mL 0.25N (0.04167M) $K_2Cr_2O_7$ solution and mix.

e. Add slowly 30mL concentrated H_2SO_4 containing Ag_2SO_4 mixing thoroughly. This slow addition along with swirling prevents fatty acids to escape due to generation of high temperature. Alternatively attach flask to condenser with water flowing and then add H_2SO_4 slowly through condenser to avoid escape of volatile organic substance due to generation of heat.

f. Mix well. If the colour turns green, either take fresh sample with lesser aliquot or add more potassium dichromate and acid.

g. Connect the flask to condenser. Mix the contents before heating. Improper mixing will result in bumping and blow out of flask content.

h. Reflux for a minimum of 2 hours. Cool and then wash down condenser with distilled water.

i. Disconnect reflux condenser and dilute the mixture to about twice its volume with distilled water. Cool to room temperature and titrate excess $K_2Cr_2O_7$ with0.1M FAS using 2-3 drops of ferroin indicator. The sharp colour change from blue green to reddish brown indicates end-point or completion of the titration. After a small time gap, the blue-green colour may reappear. Use the same quantity of ferroin indicator for all titrations.

j. Reflux blank in the same manner using distilled water instead of sample.

Alternate procedure for low COD samples less than 50mg/L: Follow similar procedure with two exceptions (i) use standard 0.025N (0.004167M) $K_2Cr_2O_7$ and (ii) titrate with standardize 0.025M FAS. The sample volume should be 5.mL. Exercise extreme care with this procedure because even a trace of organic matter on the glassware or from the atmosphere may cause gross errors. Compute amount of $HgSO_4$ to be added based on chloride concentrations. Carry blank reagent through the same procedure.

14.7 Calculations :-

COD as mg/L = (a b) x N x 8000 / mL sample

Where, a = mL FAS used for blank

b = mL FAS used for sample

N = normality of FAS

8000 = Milieq. wt. of O_2 x 1000

14.8 Precision and Bias

Precision and bias: A set of synthetic samples containing potassium hydrogen phthalate with a COD of 200mg/L was analysed in many labs with standard deviation of 13mg/L in absence of chloride.
Sources of Error:

a. The largest error is caused by using a non-homogeneous sample. Every effort should be made to blend and mix the sample so that solids are never excluded from any aliquot.

b. Use volumetric flasks and volumetric pipettes with a large bore.

c. The $K_2Cr_2O_7$ oxidising agent must be precisely measured. Use a volumetric pipette and use the same one each time if possible.

d. When titrating, be certain that the burette is clean and free of air bubbles.

e. Always read the bottom of the meniscus and position the meniscus of eye level.

Carry distilled water blank through a same procedure to nullify impurities if any. A standard solution of glucose or potassium acid phthalates should be checked for precision and accuracy every fortnight. Duplicate analysis is preferred.

Method Sensitivity:

- Standard procedure is precise and accurate for COD of 50mg/L or more
- For low COD more sample volume and the dilute reagents are used
- Interference by chloride needs to be handles very carefully to get accurate results

14.9 Interferences

Fatty acids, straight chain aliphatic compounds, aromatic hydrocarbons, chlorides, nitrite and iron interfere in the estimation. The interference caused by chloride can be eliminated by the addition of mercuric sulphate to the sample prior to the addition of other reagents. About 1.0g of mercuric sulphate is adequate to complex 100mg chloride ions in the sample in the form of poorly ionized soluble mercuric chloride complex. Addition of Ag_2SO_4 to concentrated H_2SO_4 as a catalyst stimulates the oxidation of straight chain aliphatic and aromatic compounds. Nitrite nitrogen exerts a COD of 1.14mg/mg NO_2-N.

Sulphamic acid at the rate of 10mg/mg NO_2-N may be added to $K_2Cr_2O_7$ solution to avoid interference caused by NO_2-N. Aromatic hydrocarbons and pyridine are not oxidised under any circumstances. Volatile organic compounds will react in proportion to their contact with the oxidant. For complete oxidation of organic matter, it is necessary to take volumes of Sulphuric acid and sample plus potassium dichromate in 3:2:1 ratio. However, to maintain the ratio, the volumes and strength of oxidant/sample may suitable be varied.

14.10 Safety

In carrying out the procedures, use proper safety measures, including protective clothing, eye protection and a fume hood. Reagents containing heavy metals ($HgSO_4$ and Ag_2SO_4) should be disposed of as toxic wastes. Use of such chemicals can be minimised whenever feasible.

14.11 Pollution prevention and waste minimization

Since hazardous chemicals like silver and mercury salts, Sulphuric acid, dichromate are used

in the test, the quantity of such chemicals can be minimised by selecting minimum suitable sample size. The liquid waste generated should be treated as hazardous waste. Adequate dilution of such waste before final disposal is essential.

B. Closed reflux (titrimetric and colorimetric) method using COD digester

14.12 Principle

The closed reflux (titrimetric and colormetic) method using metallic salt reagents are more economical but require homogenization of samples to obtain reproducible results. This method is conducted with ampules and culture tubes with pre-measured reagents which are available commercially. Moreover, for performing the tests, instructions furnished by the manufacturer are to be followed. Measurement of sample volume and reagent volume are critical. This method is economical in the use of metallic salt reagents and generate smaller quantity of hazardous wastes.

The principle of oxidation reaction is similar to open reflux method. Volatile organic compounds are more completely oxidised in a closed system because of longer contact time with oxidants. Digestion vessels with premixed reagents are also available from commercial suppliers.

14.13 Apparatus and equipment

a. Digestion vessels with premixed reagents and other accessories commercially available.

b. Borosilicate culture tubes 16 x 100 mm, 20 x 150 mm or 25 x 150 mm with TF and lined screw caps

c. Borosilicate ampule 10mL cap 19 to 20 mm diameter

d. Block heater to operate at 150 ± 2°C with holes to accommodate digestion vessels. Care for culture tube caps required

e. Micro-burette

f. Ampule sealer

14.14 Reagents and standards

Standard potassium dichromate digestion solution 0.01667M: Dissolve 4.903 g $K_2Cr_2O_7$, primary standard grade, previously dried at 150°C for 2 hour in 500mL distilled water, add 167mL conc. H_2SO_4 at the rate of 5.5g Ag_2SO_4/kg H_2SO_4. Let stand 1 to 2 d to dissolve and mix.

Ferroin indicator solution: Dissolve 1.485g 1, 10-phenanthroline monohydrate and 695 mg $FeSO_4.7H_2O$ in distilled water and dilute to 100mL. This indicator solution may be purchased already prepared.

Standard ferrous ammonium sulphates (FAS) titrant, approximately 0.1M: Dissolve 39.2g $Fe(NH_4)_2 (SO_4)_2.6H_2O$ in distilled water. Add 20mL concentrated H_2SO_4, cool and dilute to 100mL. Standardise this solution daily against standard $K_2Cr_2O_7$ solution.

14.15 Sample collection, preservation and storage

Refer section 14.4.

14.16 Calibration

Dilute 5mL standard $K_2Cr_2O_7$ digestion mixture to about 100mL. Titrate with FAS using 0.1 to 0.15mL. (2 to 3 drops) ferroin indicator.

Normality of FAS solution

$$= \frac{\text{Volume of 0.01667 M } K_2Cr_2O_7 \text{ solution treated, mL}}{\text{Volume FAS used in titration, mL}} \times 0.1$$

14.17 Procedure

a. Treatment of sample with COD of > 50mg/L

b. Blend sample if suspended matter is present

c. Wash culture tubes and caps with 20% H_2SO_4 before first use

d. Refer the following to select analytical parameters for proper sample and reagent volume

e. Place sample in culture tube or ampule

f. Add digestion mixture

g. Carefully run sulphuric acid reagent down inside of vessel

h. Tightly cap the tubes or seal ampules. Invert several times for proper mixing

i. Place tubes or ampules in preheated reaction block digestor

j. Reflux for 2h at 150°C behind a protective shield

k. Cool to room temperature

l. Remove caps and put TFE covered magnetic stirrer

m. Titrate while stirring with FAS using 1 or 2 drops of ferrous indicator

n. The end points is from blue-green to reddish brown

o. Reflux and titrate blank in similar way with distilled water

Standard ferrous ammonium sulphates (FAS) titrant, approximately 0.1M: Dissolve 39.2g $Fe(NH_4)_2 (SO_4)_2.6H_2O$ in distilled water. Add 20mL concentrated H_2SO_4, cool and dilute to100mL. Standardise this solution daily against standard $K_2Cr_2O_7$ solution.

14.15 Sample collection, preservation and storage

Refer section 14.4.

14.16 Calibration

Dilute 5mL standard $K_2Cr_2O_7$ digestion mixture to about 100mL. Titrate with FAS using 0.1 to 0.15mL. (2 to 3 drops) ferroin indicator.

Normality of FAS solution

$$= \frac{\text{Volume of 0.01667 M } K_2Cr_2O_7 \text{ solution treated, mL}}{\text{Volume FAS used in titration, mL}} \times 0.1$$

14.17 Procedure

a. Treatment of sample with COD of > 50mg/L
b. Blend sample if suspended matter is present
c. Wash culture tubes and caps with 20% H_2SO_4 before first use
d. Refer the following to select analytical parameters for proper sample and reagent volume
e. Place sample in culture tube or ampule
f. Add digestion mixture
g. Carefully run sulphuric acid reagent down inside of vessel
h. Tightly cap the tubes or seal ampules. Invert several times for proper mixing
i. Place tubes or ampules in preheated reaction block digestor
j. Reflux for 2h at 150°C behind a protective shield
k. Cool to room temperature
l. Remove caps and put TFE covered magnetic stirrer
m. Titrate while stirring with FAS using 1 or 2 drops of ferrous indicator
n. The end points is from blue-green to reddish brown
o. Reflux and titrate blank in similar way with distilled water

Table 14.1: I Sample and reagent quantities for various digestion vessels

Digestion Vessel	Sample mL	Digestion Solution mL	Sulphuric Acid Reagent mL	Total Final Volume mL
Culture Tubes:				
16 x100 mm	5.00	1.50	3.5	7.5
20 x 150 mm	2.50	3.00	7.0	15.0
25 x 125 mm	10.00	6.00	14.0	30.0
Standard10-mL Ampules	2.50	1.50	3.5	7.5

14.18 Calculation

COD as mg O_2/L = (A B) x M x 8000 / ml sample

Where:

A = mL FAS used for blank

B = mL FAS used for sample

M = molarity of FAS, and

8000 = milli equivalent weight of oxygen x 1000 mL/L

14.19 Precision and Bias

A set of synthetic containing potassium hydrogen phthalate and NaCl was tested by 74 laboratories. At a COD of 200 mg O_2/L in the absence of chloride, the standard deviation was ± 13 mg/L (coefficient of variation, 6.5%). At COD of 160 mg O_2/L and 100 mg Cl/L, the standard deviation was ± 14 mg/L (coefficient of variation, 10.8%).

For quality control refer Open Reflux Method.

a. Preferable analyse duplicate samples
b. Proper homogenisation is essential for reproducible results
c. Make volumetric measurement as accurate as possible
d. Use class-A volumetric flask

14.20 Interferences

Refer section 14.9.

14.21 Safety

Refer section 14.10.

14.22 Pollution prevention and waste management

Refer section 14.11.

14.23 Bibliography

1. Standard Methods for the Examination of Water and Wastewater; APHA, AWWA AND WEF, 21st Edition, 2005.
2. Clair N. Sawyer, Perry Z. McCarty. Chemistry for Environmental Engineering, 4th Edition, Gener F. Parkin.
3. American Society for Testing and Materials, (1195). Standard Methods for Chemical Oxygen Demand (Dichromate Oxygen Demand of Water D1252-95, ASTM Annual Book of Standard, American Testing and Materials, Philadelphia, PA.

15 Total Organic Carbon (TOC)

Introduction:

The Total Organic Carbon (TOC) estimate provides speedy way of estimating organic carbon but does not permit to differentiate between biologically and chemically oxidisable portion of organic compounds.

The organic carbon in water and wastewater is composed of a variety of organic compounds in various oxidation states. Some of these carbon compounds can be oxidized by biological or chemical processes Therefore, biochemical oxygen demand (BOD) and chemical oxygen demand (COD) are used conventionally. TOC is a more convenient and direct expression of total organic content. If a repeatable empirical relationship is established between TOC and BOD or COD, then TOC can be used to estimate the equivalent BOD or COD. The relationship must be established independently, for each set of matrix conditions, such as various points in a treatment process. Unlike BOD or COD, TOC is independent of the oxidation state of the organic matter and does not measure other organically bound elements, such as nitrogen and hydrogen, and inorganics that can contribute to the oxygen demand measured by BOD and COD. Hence, TOC measurement does not replace BOD and COD testing.

A. Combustion-infrared method

15.1 Principle

High temperature combustion method is suitable for raw water source for water treatment plants, with low turbidity and smaller particle size of suspended matter. Depending on the type of instrument, injection part and needle, homogenisation is required to be performed to avoid clogging and achieve repeatability of analysis. Instrumental TOC method utilises high temperature, catalysts and oxygen or lower temperature (<100°C) with irradiation, chemical oxidants, or combinations of these oxidants to convert organic carbon to carbon dioxide (CO_2). The CO_2 may be measured directly by a non-dispersive infrared analyser or CO_2 may be titrated chemically. In most water samples, the inorganic carbon (IC) fraction is many times greater than organic fraction. Eliminating or compensating for IC interferences requires multiple determinations to measure TOC. IC interference can be eliminated by acidifying samples to pH 2 or less to convert IC species to CO_2. Subsequently, purging the sample with a purified gas removes the CO_2 by volatilization. Alternatively, IC interference may be compensated for by separately measuring total carbon (TC) and inorganic carbon (IC). The difference between TC and IC is TOC.

A homogenized micro-portion of the sample in injected into a heated reaction chamber packed with a suitable catalyst, e.g. cobalt oxide, platinum group metals or barium chromate. The water is vaporized and the organic carbon is oxidized to CO_2 and H_2O. The CO_2 from oxidation of organic and inorganic carbon is transported in the carrier-gas streams and is measured by means of a non-dispersive infrared analyser. Where TC is measured, IC should be measured separately and TOC is obtained by difference. Measure IC by injecting the sample into a separate reaction chamber packed with phosphoric acid-coated quartz beads. Under the acidic conditions, all IC is converted to CO_2 which is measured by detector. Under these conditions, organic carbon in not oxidised and only IC is measured. Alternatively, convert inorganic carbonates to CO_2 with acid and remove the CO_2 by purging before sample injection.

15.2 Apparatus and equipment

a. Total organic carbon analyser
b. Syringes: 0 to 50µL, 0 to 200µL, 0 to 500µL, and 0 to 1mL
c. Sample blender or homogenizer
d. Magnetic stirrer and TFE-coated stirring bars
e. Filtering apparatus and 0.45µm-pore diameter filters

15.3 Reagents and standards

a. Reagent water: Prepare blanks and standard solutions from carbon free water, preferably use carbon-filtered, redistilled water.
b. Phosphoric acid (H_3PO_4) Conc.
c. Organic carbon stock solution: Dissolve 2.1254 g anhydrous potassium biphthalate ($C_8H_5KO_4$), in carbon-free distilled water and dilute to 1000mL; 1.00mL = 1.00mg carbon. Alternatively, use any other organic-containing compound of adequate purity, stability and water solubility. Preserve by acidifying with H_3PO_4 or H_2SO_4 to pH<2.
d. Inorganic carbon stock solution: Dissolve 4.4122 g anhydrous sodium carbonate (Na_2CO_3) in water, add 3.497 g anhydrous sodium bicarbonate ($NaHCO_3$) and dilute to 1000mL; 1mL = 1mg carbon. Alternatively use any other inorganic carbonate compound of adequate purity, stability and water solubility. Keep tightly stoppered. Do not acidify.
e. Carrier gas: Purified oxygen or air, CO_2- free and containing less than 1ppm hydrocarbon (as methane).
f. Purging gas: Any gas free of CO_2 and hydrocarbons.
g. Sample preparation: If dissolved TOC is to be estimated, filter the sample through 0.45µm pore dia filter.
h. If the sample is turbid or colloidal or oily, homogenize it in blender until satisfactory replication is obtained.

15.4 Sample collection, and preservation and storage

Collect and stored samples in glass bottles properly cleaned and rinsed with organic free water, protected from sunlight and sealed with TF and backed septa. Preserve the samples by holding at 4°C for maximum period of 7 days.

15.5 Calibration

The working standards are prepared for organic and inorganic carbon from stock standards. Calibration curve is plotted and stored in the instrument which is used to calculate the concentrations.

15.6 Procedure

a. Follow manufacture's instructions or analyser assembly, testing calibration and operation
b. The latest instrument provides the direct value of TC/IC in mg/L
c. Take standard solutions in the suitable range
d. Inject standards for calibration curve
e. Run a blank
f. Vary the sample size form 20 to 100µL
g. To free the sample from inorganic carbon, reduce its pH to 2 and pass CO_2 free N_2 gas
h. Take sample from this by a capillary syringe and inject into the analyser or follow method of injection suggested by manufacturers
i. Measure the response and repeat with three such estimations to chain an average
j. Multiply the value obtained by dilution factor, if applied
k. Regularly analyse laboratory control samples/standards to confirm performance.

15.7 Calculations

The instrument is calibrated with a series of standards for organic and inorganic carbon to cover the expected range. The instrument provides the values for TC and IC for samples. Calculate TOC by subtraction. Express the results in mg/L.

15.8 Precision and Bias

Precision: Laboratory studies of high temperature combustion methods should be conducted

with known addition method on replicate samples to get precision and accuracy. The difficulty of sampling particulate matter on unfiltered samples limits the precision of the method to approximately 5 to 10%. Minimum detectable concentration is 1mg carbon/L. This can be achieved with most combustion-infrared analysers although instrument performance varies.

Guidelines value: No guideline value is prescribed, but TOC of drinking water ranges from 100µg/L to > 25000µg/L. For drinking water, organic compounds may react with disinfectants to produce potentially toxic and carcinogenic compounds.

Method sensitivity: Range 1-150mg/L.

TOC up to ppb level can be estimated with high sensitivity catalyst. Minimum detectable limit of 1 mg/L can be lowered by concentration of sample, increasing portion of sample taken for injection or considering high sensitivity catalyst.

15.9 Interference

Removal of carbonate and bicarbonate by acidification and purging with purified gas results in the loss of volatile or organic substances. The volatiles also can be during sample blending, particularly if the temperature is allowed to rise. Another important loss can occur if large carbon-containing particles fail to enter the needle used for injection. Avoid contaminated glassware, plastic containers and rubber tubing. Analyse reagent blank following the same treatment procedure.

15.10 Safety

The normal safety measures are required to be adopted while handling heavily polluted samples and chemicals. The manufacturer's instructions for starting and closing of equipments should strictly be followed for proper maintenance of instrument and its part like catalyst, detectors etc.

15.11 Pollution prevention and waste minimization

No special precautions are required as no chemicals are added and basically it is instrumental method of analysis.

15.12 Bibliography

1. Standard Methods for the Examination of Water and Wastewater; AOHA, AWWA and WEF, 21st Edition, 2005.

16 Total Solids

Introduction:

The term 'solid' refers to the matter either filtrable or non-filtrable that remains as residue upon evaporation and subsequent drying at a defined temperature. Further categorisation depends upon depends upon the temperature employed for drying and ignition. Different forms of solids are defined on the basis of method applied for their determination. Solids may affect water or effluent quality adversely in number of ways. Water with high dissolved solids may include an unfavourable physiological reaction in the transient consumer and generally are of inferior palatability. Highly mineralized waters are unsuitable for many industrial applications. High suspended solids in waters may be aesthetically unsatisfactory for such purposes as bathing. Analysis of total solids are important to decide upon the various unit operations and processes in physical and biological wastewater treatment and to asses its performance evaluation. For assessing compliance with regulatory agency, wastewater effluent limitations for various forms of solids act as indicating parameters.

A. Total solids

16.1 Principle

Residue left after the evaporation and subsequent drying in oven at specific temperature 103-105°C of a known volume of sample are total solids. Total solids include "Total suspected solids" (TSS) and "Total dissolved solids" (TDS). Whereas loss in weight on ignition of the same sample at 500°C, 50°C, in which organic matter is converted to CO_2 volatilisation of inorganic matter as much as consistent with complete oxidation of organic matter, are volatile solids.

16.2 Apparatus and equipment

a. Electrically heated temperature controlled oven
b. Monopan balance
c. Evaporating dish (200mL)
d. Pipettes
e. Measuring cylinder (100mL)

16.3 Sample collection, preservation and storage

The water samples may be collected in resistant glass or plastic bottle. Water has considerable solvent property. There is possibility of increase in mineral content of sample, if water is collected and stored in non-resistant glass bottle. The effect is pronounced with alkaline water. Exclude particles such as leaves, sticks, fish and lump of faecal matter in the sample. Begin analysis as soon as possible due to impracticality of preservation of sample.

16.4 Calibration

The oven thermometer and balance need to be properly calibrated regularly.

16.5 Procedure

a. Take a known volume of a well-mixed sample in a tarred dish ignited to constant weight (W_1)

b. Evaporate the sample to dryness at 103-105°C for 24hrs.

c. Cool in desiccator, weigh and record the reading (W_2)

d. Ignite the dish for 15-20 minutes in a muffle furnace maintained at 550±50°C.

e. Cool the dish partially in air until most of heat has been dissipated, and then transfer to a desiccator for final cooling in a dry atmosphere and record final weight (W_3).

f. The concentration is to be calculated in percent by weight.

16.6 Calculation

The total and the volatiles solids are expressed as:

Total solids, mg/L = $(W_2 - W_1) \times 1000$ / mL of sample

and

$(W_2\ W_3) \times 1000$ / mL of sample

Where W_1, W_2 and W_3 are recorded in mg.

16.7 Precision and Bias

The precision of the method is about ± 5%.

The result for total, volatile and fixed residues are subject to considerable error because of volatile compounds during evaporation of carbon dioxide and volatile during ignition, an

also because of the presence of calcium oxide in the ash. Results for residues high in oil or grease content may be of questionable value because of the difficulty of drying to constant weight in a reasonable time. By definition, results will not include materials that are volatile under the conditions of the procedure.

In the interpretation of results, these possible sources of error must be recognized. The temperature at which the residue is dried has an important bearing on the results, because weight losses due to volatilisation of organic matter, mechanically occluded water, water of crystallization and gases fro, heat-induced chemical decomposition, as well as weight gains due to oxidation, depend on the temperature and the period of heating. A choice of drying temperatures is provided and the analyst should be familiar with the probable effects of each. The analysis should be performed in duplicate to check the precision of method. Single laboratory duplicate analysis of 41 samples of water and wastewater showed standard deviation of difference of 6 mg/L.

B. Total dissolved solids

The filterable residue is the material that passes through a standard glass filter disk and remains after evaporation and drying at 180°C.

16.8 Apparatus and equipment

Evaporatory dish (porcelain) 100/200mL

Drying oven equipped with thermostatic control capable of maintaining the temperature within 2°C range.

Desiccator provided with desiccants

Analytical balance 200mg capacity of weighing to 0.1mg

Filter holder Gooch crucible adapter or membrane filters

Suction flask 500mL capacity

16.9 Sample collection, preservation and storage

Refer section 16.3. Begin analysis as soon as possible due to impractically of preservation of sample.

16.10 Procedure

Filter the well-mixed sample under vacuum through membrane filter or Gooch Crucible. Transfer 100mL or more, depending upon the concentration of dissolved solids, in a weighed evaporating dish.

Evaporate to dryness on steam bath. Dry the evaporated sample for at least 1 hour in an oven at 180±2°C. Cool in a desiccator and weigh. Repeat the drying until constant weigh is obtained or weight loss is less than 0.5mg.

16.11 Calculation

mg/L total filtrable residue at 180°C = (A B) x 1000 / C

Where:

A = weight of dried residue + dish

B = weight of dish

C = mL of filtrate used

16.12 Precision and Bias

The analysis should be performed in duplicate to check the precision of method. A synthetic unknown sample with 134 mg/L filterable residue analysed at different laboratories at the temperature of 103-105°C showed standard deviation of ± 13mg/L.

16.13 References

1. Howard, C.S., (1933). Determination of total dissolved solids in water analysis. Ind. Engg. Chem. Anal. Ed., 5:4.
2. Standard Methods for the Examination of Water and Wastewater, APHA, AWWA and WEF, 21st Edition, 2005.

17 Turbidity

17.1 Principle

Turbidity can be measured by its effect on the scattering light, which is termed as Nephelometry. Turbidimeter can be used for sample with moderate turbidity and nepelometer for sample with low turbidity. Higher the intensity of scattered lights higher the turbidity.

Turbidity is an expression of the optical property that causes light to be scattered and absorbed rather than transmitted in straight lines through the sample. The standard method for the determination of turbidity has been based on the Jackson candle turbidity meter. However, the lowest turbidity value that can be measured directly on this instrument is 25 units. An indirect method is necessary to estimate the turbidity in the range of 0-5 units; the turbidities of treated water generally fall in this range. Most commercial turbidimeters available for measuring low turbidities give comparatively good indicators of the intensity of light scattered in one particular direction, predominantly at right angle to the incident light. These nephelometers are relatively unaffected by small changes in design parameters and are therefore specified as the standard instrument for measurement of low turbidities. Results from nephelometric measurements are expressed as nepholometric turbidity units (NTU).

17.2 Sample collection, preservation and storage

Determine turbidity on the day the sample is taken. It storage is unavoidable, store the samples in dark for up to 24 hr. Prolonged storage before measurement is not recommended because irreversible changes in turbidity may occur. All samples should be shaken vigorously before examination.

17.3 Calibration

Prepare calibration curves in the range 0-400 units by carrying our appropriate dilutions of solutions III and IV above and taking reading on turbidimeter.

17.4 Precision and Bias

For comparison of water treatment efficiencies, it may be desirable to estimate turbidity more closely. However, the uncertainties and discrepancies in turbidity measurements make it unlikely that two or more laboratories will duplicate results on the same sample more closely than specified. To maintain the precision, analyse the sample in duplicate.

18

Phosphate (PO_4^{3-})

Introduction:

Phosphorous occurs in natural waters and in wastewater almost solely in the form of various types of phosphates. These forms are commonly classified into orthophosphates and total phosphates. These may occur in the soluble form, in particles of detritus or in the bodies of aquatic organisms.

The various forms of phosphates find their way into wastewater, effluents and polluted water from a variety of sources. Larger quantities of the same compounds may be added when the water is used for laundering or other cleaning, since these materials are major constituents of many commercial cleaning preparations. Orthophosphates applied to agricultural or residential cultivated land as fertilizers are carried into surface waters with storm runoff and to a lesser extent with melting snow. Organic phosphates are formed primarily by biological processes. They are contributed to sewage by body wastes and food residues.

Presence of phosphates in water and wastewater analysis has a great significance. Phosphate in small concentration are used in water supplies to reduce scale formation, to increase carrying capacity of mains, to avoid corrosion in water mains, to remove iron and manganese in micro quantities and in coagulation especially in acid conditions. The presence of phosphate in large quantities in fresh waters indicates pollution through sewage and industrial wastes. It promotes growth of nuisance causing micro-organisms. Though phosphate posseses problems in surface waters, its presence is necessary for biological degradation of wastewaters. Phosphorus is an essential nutrient for the growth of organisms and helps for the primary productivity of a body of water.

A. Stannous chloride method

Phosphate analysis embodies two general procedural steps: (a) conversion of the phosphorus form of interest to soluble orthophosphate separation of phosphorus into its various forms is largely analytically defined but the analytical differentiations have been selected so that they may be use for interpretive purposes.

Separation of "filterable" from "particulate" forms of phosphate depends on filtration through 0.45-µm membrane filter. The selection of membrane filtration is made because of the greater likelihood of obtaining a consistent separation of particle sizes by the membrane filtration technique. Pre-filtration through a glass fibre may be used to hasten the filtration rate.

Acid hydrolysis at boiling-water temperature is designed to convert filterable and particulate condensed phosphates to filterable orthophosphate. The hydrolysis unavoidably releases some phosphate from organic compounds, but this factor has been reduced to a minimum by judicious selection of acid strength and hydrolysis time and temperature. The term "acid hydrolysable phosphate" is preferred over "condensed phosphate" technique.

The phosphate fractions that are converted to orthophosphate only by oxidative destruction of the organic matter presence are considered "organic" or "organically bound" phosphate. The severity of the oxidation required for this conversion depends on the form and to some extent on the amount of the organic phosphate which occurs both in the filterable and in the particular fractions.

18.1 Principle

In acidic condition, orthophosphate reacts with ammonium molybdate to form molybdophosphoric acid. It is further reduced to molybdenum blue by adding reducing agent such as stannous chloride or ascorbic acid. The blue colour developed after addition of ammonium molybdate is measured at 690 or 880nm within 10-12 minutes after development of colour by using blank. The concentration is calculated from the standard graph. The intensity of the blue coloured complex is measured which is directly proportional to the concentration of phosphate present in the sample.

18.2 Apparatus and equipment

a. Colorimeter for use at 690nm and 880nm providing 0.5cm light path.

b. Nessler tubes, 100mL.

18.3 Reagents and standards

a. Stock phosphate solution: Dissolve 219.5mg anhydrous KH_2PO_4 in distilled water and dilute to 1000mL. 1mL = 50mg $PO_4^{3-}P$

b. Phosphate working solution: Dilute 50mL stock solution to 1000mL with distilled water. 1mL = 2.50mg $PO_4^{3-}P$

c. Ammonium molybdate solution: Dissolve 25g in about 175 mL distilled water. Add carefully 280mL conc H_2SO_4 to 400mL distilled water. Cool and add molybdate solution and dilute to 1000mL.

d. Strong acid reagent: Add 300mL conc. H_2SO_4 to 600mL distilled water. Add 4mL conc. HNO_3, cool, dilute to 1000mL.

e. Sodium hydroxide 6N: Dissolve 24g NaOH and dilute to 100mL.

f. Phenolphthalein indicator: Dissolve 0.5g in 500mL 95% ethyl alcohol. Add 500mL distilled water. Add drop-wise 0.02 N NaOH till faint pink colour appears (pH 8.3).

g. Stannous chloride reagent I: Dissolve 2.5gm fresh $SnCl_2.H_2O$ in 100mL glycerol. Heat on water bath to ensure complete dissolution.

h. Dilute stannous chloride reagent II: Mix 8mL stannous chloride reagent I with 50mL glycerol and mix thoroughly.

i. Potassium antimonyl tartrate solution: Dissolve 2.7g in 800mL distilled water and dilute to 1000mL.

j. Ascorbic acid: Dissolve 1.76g ascorbic acid in 100mL distilled water. The solution is stable for a week at 4°C.

k. Combined reagent: Mix 250mL, 5N sulphuric acid, 75mL ammonium molybdate solution and 150mL ascorbic acid solution. Add 25mL potassium antimonyl tartrate solution and mix well. The solution must be prepared daily.

18.4 Sample collection, preservation and storage

The sample should be collected in glass/polythene container. Conventional sampling practice should be followed. The preservation of sample should be done as per the procedure given in chapter 1.

18.5 Calibration

a. Into a series of 100mL Nessler tubes pipette appropriate amounts of phosphate working solution to cover the range of 5-30mg/L or 0.3-2mg/L P when $SnCl_2$/Ascorbic acid reagent is used as a reducing agent.

b. Add 4mL ammonium molybdate followed by 0.5mL stannous chloride or 8mL combined reagent and dilute to 100mL with distilled water and mix well. Allow to stand for 10 minutes.

c. Prepare blank using distilled water in the same way.

d. Measure the intensity of blur coloured complex at 690nm or 880nm between 10 and 12 minutes after the development of the colour.

e. Plot absorbance vs. phosphate concentration to give a straight line passing through the origin.

18.6 Procedure

Orthophosphate: Take suitable volume of the sample in a Nessler tube and continue according to the procedure described for the preparation of calibration curve. From the calibration curve, compute the concentration of phosphate in the volume of sample taken. Calculate and express the result in mg/L PO_4^{3-}-P.

Total Acid Hydrolysable Phosphate (Total inorganic phosphate): Take suitable volume of the

sample in a conical flask. Add 1drop of phenolphthalein indicator. Add strong acid reagent till pink colour disappears. Add 1mL in excess. Boil for 5 minutes, cool, filter if necessary. Transfer it to Nessler tube and neutralize to phenolphthalein with NaOH. Now proceed as described in the preparation of calibration curve. Measure the intensity of the complex at 690nm or 880nm and read the corresponding concentration from the calibration curve.

The result indicates the sum of all inorganic phosphate, i.e., the orthophosphate originally present in the sample, and the acid-hydrolysable phosphate. It can, therefore, be recorded as total inorganic phosphate. The amount of acid hydrolysable phosphate is obtained by subtracting the orthophosphate from the result.

Total phosphate: Organically combined phosphorus and all phosphate including polyphosphate as first converted to orthophosphate by digestion as given below.

Take 100mL well mixed sample in 150mL conical flask. Add one drop of phenolphthalein indicator. If red colour develops, add sulphuric acid solution dropwise to just discharge the colour. Then add 1mL sulphuric acid solution in excess. Boil gently for at least 90 minutes, adding distilled water to keep the volume between 25 and 50mL. Cool, add one drop phenolphthalein indicator and neutralize to a faint pink colour with hydroxide solution. Filter if necessary and restore the volume to 100mL to 50mL. Now proceed as described in the preparation of calibration curve. Read the concentration of phosphate from the calibration curve.

For those samples which are difficult to get clear with the above digestion, the (i) Perchloric acid digestion (ii) Sulphuric acid-nitrite acid digestion (iii) Per-sulphate digestion procedure may be followed which are in the decreasing order of rigour. Refer acid digestions in the metal analysis.

Filtrable (dissolved) forms of phosphates: Samples for the determination of filtrable (dissolved) orthophosphate, filtrable acid hydrolysable phosphate and total filtrable phosphates should be filtered through membrane filters of 0.45µm pore size and estimate as per the procedure given above.

Particulate phosphate: Particulate phosphate is usually calculated by subtracting the filtrable phosphate from the total phosphate.

18.7 Calculation

The concentration is calculated from the standard calibration curve.

18.8 Precision and Bias

The analysis of sample in different laboratories showed relative standard deviation and relative error percentage, a shown in Table 18.8: I.

Table 18.8: I: Method performance in phosphate determination

Sl. No.	Concentration mg/L	RSD	RE
1	100	75.2	21.6
2	600	19.6	21.6
3	7000	8.6	5.4

RSD: Relative standard deviation
RE: Relative error

For quality assurance sample should be analysed in duplicate. Run parallel check with standards in the concentration range of sample for quality control.

18.9 Interferences

Arsenates in concentration of 100mg/L react with the molybdate reagent to produce a blue colour similar to that formed with phosphate. Silica in large concentration up to 10mg/L interferes with the test. Presence of hexavalent chromium and $NO2^-$ produce low results. $NO2^-$ interference is avoided by adding sulphuric acid to the sample prior to addition of ammonium molybdate, whereas chromium interference can be removed by adding ascorbic acid (1mL) before standard procedure.

18.10 Pollution prevention and waste management

The chemicals used in analysis are in micro-quantities and will be added to waste in very dilute form which will not necessarily cause any problems. However, routine hazardous waste management practices should be followed.

18.11 Reference

1. Standard Methods for the Examination of Water and Wastewater; APHA, AWWA and WEF, 21st Edition, 2000

NITROGEN (Ammonia)

19

Introduction:

Ammonia is produced by the microbiological degradation of organic nitrogenous matter. It appears, therefore, in many groundwaters as well as surface waters. Concentrations of ammonia above a certain level in water polluted either due to sewage or industrial waste is toxic to fish. The proportions of the two forms of ammonia nitrogen in surface water depend on pH.

pH	6	7	8	9	10	11
$\%NH_3$	0	1	4	25	78	96
$\%NH_4$	100	99	96	75	22	4

For accurate results, it is generally preferable to distill off ammonia from the sample, and absorb in boric acid. It is then determined either by titration or colorimetrically using Nessler reagent. Direct nesslerisation of the sample is quicker depending upon interference.

A. Nesslerisation method

19.1 Principle

Ammonia produces a yellow coloured compound when reacts with alkaline Nessler reagent, provided the sample is clarified properly. Pretreatment with $ZnSO_4$ and NaOH precipitates Ca, Fe, Mg and sulphide and removes turbidity and apparent colour. Addition of EDTA (Before Nessler reagent) or Rochelle salt solution prevents precipitation of residual Ca and Mg in the presence of alkaline Nessler reagent. The chemical reaction of the method is given below:

$$2Kr_2Hgl_4 + NH_3 + 3KOH \rightarrow (NH_2Hg_2IO) + 7Kl + 2H_2O$$

19.2 Apparatus and equipment

a. Spectrophotometer: spectrophotometer having a range of 300 to 700nm.

b. Nessler tubes or 100mL capacity volumetric flasks.

19.3 Reagents and standards

a. Zinc sulphate: dissolve 10g $ZnSO_4.7H_2O$ in distilled water and dilute to 100mL.

b. Sodium hydroxide, 6N: dissolve 24g NaOH and dilute to 100mL.

c. EDTA reagent: dissolve 50g EDTA in 60mL water containing 10g NaOH. Cool and dilute to 100mL.

d. Rochelle salt solution: dissolve 50g potassium sodium tartarate in 100mL. Remove ammonia by boiling off 30mL solution, cool and dilute to 100mL.

e. Nessler reagent: mix well 100g HgI_2 and 70g KI. Dissolve in small quantity of water. Add this mixture to a cooled solution of 160g NaOH in 500mL water. Dilute to 1000mL. Keep overnight, store supernatant in coloured bottle.

f. Standard ammonium solution: dissolve 3.819g NH_4Cl dried at 100°C in distilled water and dilute to 1000mL. Dilute 10mL of the solution to 1000mL. 1mL = 10µg NH_3.

19.4 Calibration

Prepare a calibration curve using suitable aliquots of standard solution in the range of 5 to 120µg/100mL for reference following the same procedure as 1 to5 but using the standard solution in place of sample.

19.5 Procedure

a. Take 100mL of sample. Add 1 mL $ZnSO_4$ solution and 0.4 or 0.5 mL NaOH to obtain the pH of 10.5. Allow to settle and filter the supernatant through 42 No. Whatman filter paper.

b. Take suitable aliquot of sample

c. Add 3 drops of Rochelle salt solution or 1 drop of EDTA mix well.

d. Add 3mL Nessler reagent if EDTA is used or 1mL if Rochelle salt solution is used. Make up to 100mL.

e. Mix well and read percent transmission after 10 minutes at 410nm using a blank prepared in the same way by taking distilled water instead of sample.

19.6 Calculation

The concentration may be obtained directly from standard graph.

19.7 Precision and Bias

Six unknown samples with concentration 0.2mg/L-1.5mg/L, when analysed in different

laboratories showed relative standard deviation 5.3%-22%. The relative error was 1.2%-8.3%. The standard deviation and relative error decreased with increasing concentration. For quality assurance run the sample in duplicate.

19.8 Interferences

Colour, turbidity, Ca, Mg salts and Fe in the sample constitute the prime sources of interferences.

19.9 Pollution prevention and waste management

Refer section 26.10.

19.10 Bibliography

1. Standard Methods for the Examination of Water and Wastewater; APHA, AWWA and WEF, 21st Edition, 2005.

20 Nitrogen (Nitrate) (NO_3^-)

Introduction:

Determination of nitrate (NO_3^-) is difficult because of the relatively complex procedures required, the high probability that interfering constituents will be present and the limited concentration ranges of the various techniques. Nitrate is the most highly oxidised form of nitrogen compounds commonly present in natural waters. Significant sources of nitrate are chemical fertilizers, decayed vegetable and animal matter, domestic effluents, sewage sludge disposal to land, industrial discharge, leachates from refuse dumps and atmospheric washout. Depending on the situation, these sources can contaminate streams, rivers, lakes and ground water. Unpolluted natural water contains minute amounts of nitrate. Excessive concentration in drinking water is considered hazardous for infants because of its reduction to nitrite in intestinal track causing methemoglobinaemia. In surface water, nitrate is a nutrient taken up by plants and converted into cell protein. The growth stimulation of plants, especially of algae may cause objectionable eutrophication.

A. UV spectrophotometer method

The method is useful for the water free from organic contaminants and is most suitable for drinking.

Measurement of the ultraviolet absorption at 220nm enables rapid determination of nitrate. The nitrate calibration curve follows Beer's law upto 11mg/L N.

Acidification with 1N hydrochloric acid is designed to present interference from hydroxide or concentrations up to 1,000mg/L as $CaCO_3$. Chloride has no effect on the determination. Minimum detectable concentration is 40µg/L NO_3 N.

20.1 Principle

Nitrate is determined by measuring the absorbance at 220nm in sample containing 1mL of hydrochloric acid (1N) in 100mL sample. The concentration is calculated from graph from standard nitrate solution in range 1-11mg/L as N.

20.2 Apparatus and equipment

a. Spectrophotometer, for use at 220nm and 275nm with matched silica cells of 1cm or longer light path.

b. Filter: One of the following is required.
   i) Membrane filter: 0.45µm membrane filter, and appropriate filter assemble
   ii) Paper: Acid-washed, ashless hard-finish filter paper sufficiently retentive for fine precipitates.
c. Nessler tubes, 50mL, short form.

20.3 Reagents and standards

a. Redistilled water: use redistilled water for the preparation of all solutions and dilutions.
b. Stock nitrate solution: dissolve 721.8mg anhydrous potassium nitrate and dilute to 1000ml with distilled water. 1mL = 100 µg N = 443µg NO_3^-.
c. Standard nitrate solution: dilute 100mL stock nitrate solution to 1000mL with distilled water. 1mL = 10µg NO_3 N = 44.3µg No_3.
d. Hydrochloric acid solution: HCl, 1N.
e. Aluminium hydroxide suspension: dissolve 125g potash alum in 1000mL distilled water. Warm to 60°C, add 55-60mL NH_4OH and allow to stand for 1h. Decant the supernatant and wash the precipitate a number of times till it is free from Cl, NO_2 and NO_3. Finally after setting, decant off as much clean liquid as possible, leaving only the concentrated suspension.

20.4 Calibration

Prepare nitrate calibration standards in the range 0 to 350µg N by diluting 1, 2, 4, 7.....35mL of the standard nitrate solution to 50mL. Treat the nitrate standards in the same manner as the samples.

20.5 Procedure

Read the absorbance or transmittance against redistilled water set at zero absorbance or 100% transmittance. Use a wavelength of 220 nm to obtain the nitrate reading and, if necessary, a wavelength of 275nm to obtain interference due to dissolved organic matter.

20.6 Calculation

For correction for dissolved organic matter, subtract 2 times the reading at 275nm from the reading at 220nm to obtain the absorbance due to nitrate. Convert this absorbance value into equivalent nitrate by reading the nitrate value from a standard calibration curve.

Nitrate N, mg/L = mg nitrate-N / mL of sample

NO_3, mg/L = Nitrate N mg/L x 4.43

20.7 Precision and Bias

Because dissolved organic matter may also absorb at 220nm and nitrate does not absorb at 275nm a second measurement can be made at 275nm to correct the nitrate value. The extent of this empirical correction is related to the nature and concentration of the organic matter and may vary from one water to another. Filtration of the sample is intended to remove possible interference from suspended particles. Analyse the sample in duplicate for quality assurance and run 1-2 standards for quality control.

20.8 Interferences

Dissolved organic matter, nitrite, hexavalent chromium and surfactants are interferences. The latter three substances may be compensated for by independent analysis of their concentrations and preparation of individual correction curves.

Organic matter can cause a positive but variable interference. The degree of interference depends on the nature and concentration of the organic matter in the sample.
Clean all glassware thoroughly and rinse to reduce the error that might result from streaks or particles on the outside of the curves, as well as traces of surfactants or dichromate cleaning solution that might adhere on the interior glass surfaces.

Treat coloured samples with aluminium hydroxide suspension or dilute to minimise colour interference.

If the sample has a high colour or is known to contain organic interferences, add 4mL $Al_2(OH)_3$ suspension/100mL sample in an Erlenmeyer flask. Swirl to mix and settle for 5 minutes. Filter through a 0.45µm membrane filter previously washed with about 200mL distilled water. To 50mL clear filtered sample, add 1mL (1N), HCl and mix thoroughly.

20.9 Pollution prevention and waste management

The disposal of waste generated from analysis is an important aspect of waste management. The chemical used in analysis is in micro-quantity (1mL) of 1N HCl in 50mL of sample which need not have a problem of waste management.

B. Phenol disulphonic acid (PDA) method

20.10 Principle

Nitrate reacts with phenol disulphonic acid and produces a nitro-derivative which in alkaline solution develops yellow colour due to rearrangement of its structure. The colour produced follows Beer's law and is intensity proportional to the concentration of NO_3 present in the sample. The chemical reaction involved in the method is given below.

20.11 Apparatus and equipment

a. Colorimetric or spectrophotometer having a range of 300-700nm.

b. Nessler tubes, capacity, 100mL.

c. Beakers, capacity, 100mL.

d. Water bath

20.12 Reagents and standards

a. Standard silver sulphate: dissolve 4.40g Ag_2SO_4 in distilled water and dilute to 1000mL, 1mL = 1mg Cl.

b. Phenol disulfonic acid (PDA): dissolve 25g white phenol in 150 mL conc. H_2SO_4. Add 75mL fuming H_2SO_4 (15% free SO_3), stir well and heat for 2h. on water bath. If fuming sulphuric acid is not available, add additional 85mL conc. H_2SO_4 to the 150mL H_2SO_4, stir well and heat for 2h.

c. Ammonium hydroxide NH_4OH conc.

d. Potassium hydroxide, 12N: dissolve 673g KOH in distilled water and make up to 1000mL with distilled water; for hard waters use liquid ammonia.

e. Stock nitrate solution: dissolve 721.8mg anhydrous potassium nitrate and dilute to 1000mL with distilled water. 1mL = µg N.

f. Standard nitrate solution: evaporate 50mL stock nitrate solution to dryness on water bath. Dissolve residue in 2mL PDA reagent and dilute to 500mL. 1mL = 10µg N.

g. EDTA reagent: run 50g EDTA with 20mL distilled water to form a paste. Add 60mL NH_4OH and mix well.

h. Aluminium hydroxide: dissolve 125g potash alum in 1000mL distilled water. Warm to 60°C, add 55-60mL NH_4OH and allow standing for 1h. Decant the supernatant and wash the precipitate a number of times till it is free from Cl^-, $NO2^-$ and $NO3^-$. Finally after setting, decant off as much clean liquid as possible, leaving only the concentrated suspension.

20.13 Sample collection, preservation and storage

The sample should be collected in glass/polythene container. Conventional sampling practice should be followed. The preservation of sample should be done as per procedure given in chapter 9. The analysis of samples with concentration 0.1mg/L, 7mg/L in different laboratories showed relative standard deviation and relative error 7.6-25.5 percent and 4.3-28.7. The standard deviation and relative error decrease with increased concentration.

Sl. No.	Concentration mg/L	RSD	RE
1	100	25.5	28.7
2	600	14.2	8.0
3	7000	7.6	4.3

RSD: Relative standard deviation
RE: Relative error

20.14 Calibration

Prepare calibration curve using suitable aliquots of standard nitrate solution in the range of 5 to 500 mg NO_3^- N/L following the above procedure.

20.15 Procedure

Colour development

a. Neutralise the clarified sample to pH 7.0
b. Take suitable aliquot of the sample in a beaker and evaporate to dryness on water bath.
c. Dissolve the residue using glass rod with 2mL phenol disulphonic acid reagent. Dilute and transfer to Nessler tubes.
d. Add 8-10mL 12N KOH. If turbidity is developed add the EDTA reagent drop-wise till it dissolves. Filter and make up to 100mL. To avoid turbidity add 10mL conc. NH_4OH instead of KOH.
e. Prepare blank in the same way using distilled water in place of sample.
f. Read the intensity of colour developed at 410nm with a light path of 1cm. Record NO_3^- as N in mg/L.

20.16 Calculation

The concentration of Nitrate is calculated from the standard calibration curve and the values are reported in mg/L.

20.17 Precision and Bias

Analysis is to be carried out in duplicate for quality assurance. Run 1-2 standards with sample for quality control.

20.18 Interferences

Chlorides and nitrite are the two main sources of interferences. Pretreatment of sample is necessary when the interfering radicals are present.'

a. Colour removal: If the sample has a colour in excess of 10 units; and 3mL aluminium hydroxide to 150mL sample. Stir well and allow setting for a few min, filtering and using the filtrate discarding the first portion of the filtrate.

b. Nitrite removal: (i) Generally, NO_2 occurs along with NH_3 and gets eliminated in the routine test due to decomposition of NO_2 and NH_4 to N_2, (ii) Oxidise NO_2 under acid condition using $KmnO_4$ and (iii) Add sulphamic acid to the sample to suppress NO_2 interference.

c. Chloride removal: Determine the Cl contents of the sample and precipitate out as AgCl by adding appropriate amount of Ag_2SO_4 solution. One should be very careful while adding Ag_2SO_4 because excess Ag will precipitate out as silver oxide when alkali is added to sample.

20.19 Pollution prevention and waste management

The chemicals used in analysis are in micro-quantities and will be added to waste in very dilute form which will not necessarily cause any problems. However, routine waste management practices should be followed.

20.20 Reference

1. Standard Methods for the Examination of Water and Wastewater; APHA, AWWA and WEF, 21st Edition, 2005.

21 Nitrogen (Nitrite) (NO_2^-)

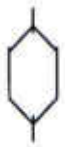
Introduction:

Nitrite in water is either due to oxidation of ammonium compounds or due to reduction of nitrate. As an intermediate stage in the nitrogen cycle, it is unstable. A usual concentration in natural water is in the range of some tenths of mg/L. Higher concentrations are present in industrial wastes, sewage and in biologically purified effluents and in polluted streams. In chlorinated supplies, levels of nitrite are often less than the limit of detection, i.e. 0.005mg/L NO_2^-N but high levels may occur in unchlorinated water. Very high nitrite levels are usually associated with water of unsatisfactory microbiological activity.

A. Colorimetric method

21.1 Principle

Nitrite (NO_2^-) is determined through formation of a reddish purple azodye at pH 2.0-2.5 by coupling diazotized sulphanilamide with N-(1-Naphthyl) ethylenediamine dihydrochloride (NED-dihydrochloride). The method is applicable to 1µg NO_2^- N/L. The colour system obeys Beer's law up to 180µg N/L with 1cm light path at 543nm. The chemical reaction involved in the method is given below:

1. $H_2SO_4 + HNO_2 + HCl \xrightarrow{\text{Azodye}}$  $+ 2H_2O$

SO_3H

Diazonium salt

2. Diazotisation Reaction

$R - NH_2 + NO_2^- + 2H^+ \longrightarrow R\text{--}N = N + 2H_2O$

Sulphanilamide

3. Coupling Reaction

$R\,N = N + -R' \longrightarrow R\,N = N\text{--}R' + H +$ [1-N-(1-Napthyl) Ethylenediamine dihydrochloride]

Where, R is $SO_2.NH_2$ and R' is $NH\,CH_2\,CH_2\text{-}NH_2$

21.2 Apparatus and equipment

1. Colorimeter or spectro-photometer that can be operated at 543nm.
2. Nessler tubes or 100mL capacity volumetric flask.

21.3 Reagents and standards

1. Sulphanilamide reagent: dissolve 5g sulphanilamide in a mixture of 50mL conc. HCl and about 300mL water. Dilute to 500mL with water. This solution is stables for many months.
2. NED-dihydrochloride solution: dissolve in 500mg N-(1-Naphthyl)-ethylenediamine dihydrochloride in 500mL water. Store in a dark bottle and replace monthly or immediately when it develops a strong brown colour.
3. Sodium oxalate (0.05N): dissolve 3.35g $Na_2C_2O_4$ in water dilute to 1000mL.
4. Stock nitrite solution: dissolve 1.2320 sodium nitrite ($NaNO_2$) in water and dilute to 1000mL; 1mL = 250µg N.
5. Standard nitrite solution: Dilute appropriate aliquot of stock nitrite solution to 1000mL with distilled water so that 1mL = 0.5 µg N in the solution.

21.4 Sample collection, preservation and storage

Most reliable are obtained in fresh sample as nitrite is an intermediate oxidation state of nitrogen, in oxidation of ammonia to nitrate and reduction of nitrate. Sample can be preserved by addition of $HgCl_2$ for a short period, if necessary. Acid preservation is not used in any case.

21.5 Procedure

1. If sample contains suspended solids, filter through a 0.45 mm pore diameter membrane filter.
2. To 50mL clear sample neutralized to pH 7 or to a portion diluted to 50mL, add 1mL sulfanilamide solution. Let reagent react for 2 to 8 min.
3. Add 1.0mL NED dihydrochloride solution and mix immediately. Measure absorbance after 10 min but before 2 h at 543 nm.
4. Prepare blank in the same way substituting water for the sample.

21.6 Calibration

1. Prepare calibration curve by pipetting suitable volume of standard nitrite solution in the range 0-180µg NO_2N/L using 1cm light path for reference.
2. Run parallel check frequently against nitrite standards preferably in the concentration range of sample. Redetermine complete calibration curve after preparing new reagents. Compute concentration of sample from calibration curves.

21.7 Precision and Bias

In a single laboratory using wastewater samples at concentrations of 0.04, 0.24, 0.55 and 1.04mg NO_3^- + NO_2^- N/L, the standard deviations were ±0.005, ±0.004, ±0.005, and ±0.01 respectively. In a single laboratory using wastewater samples at concentrations of 0.24, 0.55 and 1.04mg NO_3^- + NO_2^- -N/L, the recoveries were 100%, 102% and 100% respectively.

21.8 Interferences

Chlorine in the samples or nitrogen trichloride which normally coexists with NO_2^- produces a false colour. This can be minimised by addition of the NED dihydrochloride reagent followed by the suphanilamide acid reagent. An orange colour still may result when a substantial NCl_3 concentration is present. Under such circumstances, check for free Chlorine and NCl_3 residuals. NO_2 determinations should be carried out in filtered, turbidity-free sample.

21.9 References

1. U.S. Environmental Protection Agency (1979). Methods for Chemical Analysis of water and wastes, Method 353.3 U.S. Environmental Protection Agency, Washington, D.C.
2. Standard Methods for the Examination of Water and Wastewater; APHA, AWWA and WEF, 21st Edition, 2005.

OIL AND GREASE

22

Introduction:

Oil and grease is any material recovered as a substance soluble in petroleum ether, hexane or n-hexane. It includes other materials extracted by the solvent from an acidified sample such as sulphur compounds, certain organic dyes and chlorophyll. Oil and grease are defined by the method used for their determination. The oil and grease content of domestic industrial wastes and of sludges, is an important consideration in the handling and treatment of these materials for ultimate disposal. When treated effluents are discharged in water body, it leads to environmental degradation. Hydrocarbons, esters, oils, fats, waxes and high molecular weight fatty acids are the major materials dissolved by hexane. All these materials have a 'greasy feel'. Three methods for oil and grease estimations are (i) the partition-gravimetric method, (ii) the partition infrared method and (iii) the Soxhlet extraction method. Though methods-(i) does not provide the needed precision, it is widely used for routine analysis of samples because of its simplicity and it needs no special instrumentation and (ii) is identical to hydrocarbons. In method (iii) adequate instrumentation allows for the measurement of as little as 0.2mg oil and grease.

A. Partition-gravimetric method

22.1 Principle

Dissolved or emulsified oil and grease is extracted from water by intimate contact with n-hexane, petroleum ether (40°C/60°C) or hexane. Unsaturated fats and fatty acids oxidise readily hence precautions regarding temperature to solvent vapour displacement are included in the procedure.

22.2 Apparatus and equipment

a. Separatory funnel, 1L with TFE (Teflon) stopcock
b. Distilling flask, 125mL
c. Water bath
d. Filter paper, 110mm dia. (Whatman No. 40 or equivalent).
e. Weighing balance

22.3 Reagents and standards

a. Hydrochloric acid: HCl (1+1)

b. n-hexane

c. Petroleum ether (BP 40°C-60°C) or Hexane

d. Anhydrous sodium sulphate-Na_2SO_4

The solvent should leave no measurable residue on evaporation; distill if necessary. Petroleum ether 40°C/60°C or hexane can also be used. Plastic tubes should not be used to transfer solvent between containers.

22.4 Sample collection, preservation and storage

Collect a representative sample and preserve as per the procedure mentioned in Chapter 9. Collect separate sample for oil and grease and do not subdivide in the laboratory. Samples collected at different intervals of time may be examined individually for knowing average concentration of oil and grease. The glass bottle container should be rinsed with the solvent to remove contaminants adhered to the side walls.

22.5 Procedure

a. Collect about 1Lsample and mark sample level in bottle for later determination of sample volume. Acidify to pH 2 or lower; generally, 5mL HCl (1+1) is sufficient. Transfer to a separatory funnel. Carefully rinse sample bottle with 30mL n-hexane and add the solvent washings to separatory funnel.

b. Preferably shake vigorously for 2 min. However, if it is suspected for a stable emulsion, shake gently for 5 to 10 min.

c. Let the layers separate. Drain solvent layer through a funnel containing solvent-moistened filter paper and 10g Na_2SO_4 into a clean, tared distilling flask. If a clear solvent layer cannot be obtained and emulsion exists, centrifuge the solvent and emulsion. Transfer centrifuged material to a separating funnel and drain solvent layer through a funnel with a prerinsed filter paper and 10 g Na_2SO_4.

d. Extract twice more with 30mL solvent each but first rinse sample container with each solvent portion. Combine extracts in tared distilling flask and wash filter paper with an additional 10 to 20mL solvent.

e. Distill solvent from distilling flask in a water bath at 70°C for solvent recovery. Place flask on a water bath at 70°C for 15 min and draw air through it with applied vacuum for the final 1min after the solvent has evaporated. If the residue contains visible water, add 2mL acetone, evaporate on a water-bath and repeat the addition and evaporation until all visible water has been removed. Cool in a desiccator for 30 min and weigh.

22.6 Calculations

Total gain in weight A, of tared flask and less calculated residue B, from solvent blank is the amount of oil and grease in the sample.

Mg/L, Oil and grease= (A B) x 1000 / mL sample

Along with the results, mention the solvent used for extraction.

22.7 Precision and Bias

A recovery of about 90% with standard deviation of 0.9 mg is obtained when 14mg/L of mixture of fuel and Wesson oil is added to wastewater with 12.6 mg/L of oil and grease.

Necessary precautions are required to avoid contamination.

22.8 Interferences

No known solvent will dissolve effectively only oil and grease. Solvent removal results in the loss of short-chain hydrocarbons and simple aromatics by volatilization, and heavier residuals of some effluents may contain a significant portion of materials that are not extractable with the solvent.

22.9 Safety

Normal safety procedures should be adopted while handling organic solvents.

B. Soxhlet extraction method

22.10 Principle

Any oils and solid or viscous grease present are separated from the liquid samples by filtration. After extraction in a soxhlet apparatus with n-hexane, the residue remaining after solvent evaporation is weighted to determine the oil and grease content. Compounds volatilized at or below 103°C will be lost when the filter is dried.

22.11 Apparatus and equipment

a. Soxhlet extraction assembly
b. Vacuum pump or other source of vacuum
c. Buchner funnel, 120mm dia.
d. Electric heating mantle

e. Extraction thimble, paper

f. Filter paper, 110mm dia.

g. Muslin cloth disks, 110mm dia.

h. Water bath, capable of maintaining 70°C

i. Desiccators.

22.12 Reagents

a. Hydrochloric acid, HCl (1+1)

b. Freon (1,2,2-trichloro-1,2,2-trifluoroethane boiling point 47°C)

c. Diatomaceous-silica filter aid suspension, 10g/L distilled water

22.13 Sample collection, preservation and storage

Refer section 22.4.

22.14 Procedure

a. Collect about 1 L of sample in a wide-mouth glass bottle and mark sample level in bottle for later determination of sample volume. Acidify to pH or lower with HCl (1+1).

b. Prepare a filter consisting of a Muslim cloth disk overlaid with filter paper. Wet paper and muslin and press down edges of paper. Using a vacuum, pass 100mL filter aid suspension through prepared filter and wash with 1L distilled water. Apply vacuum until no more water passes through filter.

c. Filter acidified sample: Apply vacuum until no more water passes through filter.

d. Using forceps, transfer filter paper to watch glass. Add material adhering to edges of muslin cloth disk, collecting vessel and Buchner funnel with pieces of filter paper soaked in solvent to filter paper on watch glass.

e. Roll a filter paper containing sample and fit into a paper extraction thimble. Add any pieces of material remaining on watch glass. Wipe watch glass with a filter paper soaked in solvent and place in paper extraction thimble.

f. Dry the filled thimble in a hot air oven at 103°C for 30 min. Fill thimble with glass wool or small glass beads.

g. Weigh extraction flask. Extract oil and grease in Soxhlet apparatus, using n-hexane at a rate of 20 cycles/h for 4h.

h. Distill solvent from extraction flask in a water bath at 70°C. Place flask on a water bath at 70°C for 15 min and draw air through it using an applied vacuum for 30 min. Cool in a desiccator and weigh.

22.15 Calculations

Refer section 22.6

22.16 Precision and Bias

A recovery of about 88% with standard deviation of 1.1mg is obtained when 14mg/L of mixture of fuel and Wesson oil is added to wastewater with 12.6mg/L of oil and grease.

Necessary precautions are required to avoid contamination.

22.17 Interferences

This method is entirely empirical and duplicate results can be obtained only by strict adherence to all details. The rate and time of extraction in the soxhlet apparatus must be exactly as directed because of varying solubilities of different greases. In addition, the length of time required for drying and cooling extracted material cannot be varied. There may be a gradual increase in weigh, presumably due to the absorption of oxygen and /or gradual loss of weight due to volatilization.

22.18 Safety

Normal safety procedures should be adopted while handling organic solvents.

22.19 Pollution prevention and waste management

The waste should be treated with care to avoid contamination of traces of solvents in aqueous discharges.

22.20 Bibliography

1. Standard Methods for the Examination of Water and Wastewater, APHA, AWWA and WEF, 21st Edition, 2005.

23 Sulphate (SO_4^-)

Introduction:

Sulphate ions usually occur in natural waters. Many sulphate compounds are readily soluble in water. Most of them originate form the oxidation of sulphate ores, the solution of gypsum and anhydrite, the presence of shales, particularly those rich in organic compounds, and the existence of industrial wastes. Atmospheric sulphur dioxide formed by the combustion of fossil fuels and emitted by the metallurgical roasting processes may also contribute to the sulphate compounds of water. Sulphur trioxide (SO_3) produces by the photolytic oxidation of sulphur dioxide comes with water vapours to form sulphuric acid which is precipitated as acid rain or snow. Sulphur-bearing mineral are common in most sedimentary rocks. In the weathering process gypsum (calcium sulphate) is dissolved and sulphide minerals are partly oxidised, giving rise to a soluble form of sulphate that is carried away by water. In humid region, sulphate is readily leached from the zone of weathering by infiltrating waters and surface run off but in semiarid and arid regions the soluble salts may accumulate within a few tens of feet of land surface. Where this occurs, sulphate concentration in shallow ground water may exceed 5000mg/L and gradually decrease with depth.

Ingestion of water containing high concentration of sulphate can have a laxative effect, which is enhanced when sulphate is consumed in combination with magnesium. Water containing magnesium sulphate at levels about 1000 mg/L acts as a purgative in human adults. Taste threshold concentrations for the most prevalent sulphate salts are 200-500mg/L for sodium sulphate, 250-900mg/L for calcium sulphate, and 400-600mg/L for magnesium sulphate. Essentially on the basis of above values, which are also allied to the cathartic effect of sulphate, a guidelines value of 400mg/L is proposed. Sulphates cause scaling in water supplies, and problem of odour and corrosion in wastewater treatment due to its reduction to H_2S.

A. Turbidimetric method

23.1 Principle

This method is used for the determination of sulphate ions. Sulphate ion (SO_4^-) is precipitated in an acetic acid medium with Barium chloride ($BaCl_2$) so as to form Barium sulphate ($BaSO_4$) crystals of uniform size. The reaction involved is given below:

$Ba^{++} + SO_4 \circledR BaSO_4$ (White suspension)

Light absorbance of the $BaSO_4$ suspension is measured by a photometer or the scattering of light by Nephelometer.

23.2 Apparatus and equipment

a. Magnetic stirrer
b. Colorimeter for use at 420mm or turbidimeter/nephelometer
c. Stopwatch
d. Nessler tubes, 100mL
e. Measuring spoon 0.2 to 0.3mL

23.3 Reagents and standards

a. Buffer solution A: dissolve 30g Magnesium chloride, $MgCl_2.6H_2O$, 5g Sodium acetate $CH_3COONa.3H_2O$, 1g Potassium nitrate, KNO_3 and 20mL acetic acid, CH_3COOH (99%) in 500mL distilled water and make up to 1000mL.

b. Buffer solution B: (required when the sample sulphate (SO_4^{2-}) is less than 10mg/L). Dissolve 30g Magnesium chloride, $MgCl_2.6H_2O$, 5g sodium acetate, $CH_3COONa.3H_2O$, 1.0g of potassium nitrate, KNO_3, 0.111 g of sodium sulphate, Na_2SO_4 and 20mL acetic acid (99%) in 500mL distilled water an make up to 1000mL.

c. Barium chloride: crystals, 20-30mesh.

d. Standard sulphate solution: dissolve 0.1479g anhydrous sodium sulphate, Na_2SO_4 in distilled water and dilute to 1000mL. 1L = 100 µg SO_4

   or

   Dilute 10.4mL standard 0.02 N H_2SO_4 titrant specified in Alkalinity, Section 10.1.3, with distilled water; 1mL = 100 µg SO_4^{2-}

23.4 Sample collection, preservation and storage

In presence of organic matter certain bacteria may reduce SO_4^{2-} to S^{2-}. To avoid this, store sample at 4°C.

23.5 Calibration

Prepare standard curve by carrying standard sulphate solution through entire procedure. Space standards at 5mg/L increment in the 0 to 40 mg/L range. Read mg SO_4^{2-} resent in the sample from the standard curve.

23.6 Procedure

a. Take suitable volume of sample and dilute to 100mL into a 250mL Erlenmeyer flask

b. Add 20mL buffer solution, mix well

c. Keep the flask constantly stirred with the help of stirrer. Add 1-spatula $BaCl_2$ crystals with stirring. Continue stirring for 1 minute after addition of $BaCl_2$

d. Pour suspension into an absorption cell of photometer an measure turbidity at 5±0.5 min

e. To correct for sample colour and turbidity, run a blank to which $BaCl_2$ is not added.

23.7 Calculation

mg SO4--/L = {mg (SO_4^-) x 1000}/ {mL of sample}

Note: If buffer solution A was used, determine sulphate concentration directly from the calibration curve after subtracting sample absorbance before adding $BaCl_2$. If buffer solution B was used subtract sulphate concentration of blank from apparent sulphate concentration as determined above, because the calibration curve is not a straight line, this is not equivalent to subtracting blank absorbance from sample absorbance.

23.8 Precision and Bias

Correct the colour and turbidity present in the original sample by running blanks from which the $BaCl_2$ is not added. During preparation of standard curve, space the standards at 5mg/L increments in the 0 to 40mg/L sulphate range. Above 40mg/L the accuracy of the method decreases and the suspensions of barium sulphate lose stability. Check reliability of the calibration curve by running a standard with every three or four unknown samples. Draw the fresh calibration curve with change of fresh stalk of reagent or chemical (Barium chloride). The stirring time 1 min should be kept constant for samples and the standards.

Synthetic unknown sample containing 259mg/L sulphate, when analysed in different laboratories showed relative standard deviation of 9.1% and relative error 1.2%.

23.9 Interferences

Colour, turbidity and silica in the concentration of 500ppm interfere in this estimation. Filtration is adopted to remove colour and turbidity. In presence of organic matter, precipitation of barium sulphate may not be satisfactory.

23.10 Pollution prevention and waste management

Refer section 26.10.

23.11 References

1. ROSSUM, J.R. and P. Villarruz, (1961). Suggested methods for turbidimetric determination of sulphate in water. J. American Water Works Assoc., 53:873.
2. Standard Methods for the Examination of Water and Wastewater; APHA, AWWA and WEF, 21st Edition, 2005.

24 Sodium (Na)

24.1 Scope and application

Sodium ranks sixth among the elements in order of abundance and is present in most natural water. The levels may vary from less than 1 mg Na/L to more than 500 mg Na/L. Relatively high concentrations may be found in brines and hard water softened by the sodium exchange process. The ratio of sodium to total cations is important in agriculture and human pathology. Soil permeability can be harmed by a high sodium ratio. Persons afflicted with certain diseases require water with low sodium concentration. A limiting concentration of 2 to3 mg/L is recommended in feed water destined for high-pressure boilers. When necessary, sodium can be removed by the hydrogen-exchange process or by distillation. Sodium compounds are used in many applications, including caustic soda, sat fertilizers and water treatment chemicals.

Storage of samples:

Store samples containing low sodium concentrations or alkaline samples in polyethylene bottles to eliminate the possibility of sample contamination due to leaching of the glass container.

Methods for analysis:

A. Inductively coupled plasma method

B. Flame Emission Photometric method

A. Inductively coupled plasma method

Please refer section 28.6.A.

B. Flame emission photometric method

24.2 Summary of method

Principle: Trace amounts of sodium can be determined by flame emission photometry at the wavelength of 589 nm. The sample is sprayed into a gas flame and excitation is carried out under carefully controlled and reproducible conditions. The desired spectral line is isolated by the use of interference filters or by a suitable slit arrangement in light-dispersing devices

such as prisms or gratings. The intensity of light is measured by a phototube potentiometer or other appropriate circuit. The intensity of light at 589 nm is approximately proportional to the concentration of the element. If alignment of the wavelength dial with the prism is not precise in the available photometer, the exact wavelength setting, which may be slightly more or less than 589 nm, can be determined from the maximum needle deflection and then used for the emission measurements. The calibration curve may be linear but has a tendency to levels off at higher concentrations.

Interference: Flame photometers operating on the internal standard principle may require adding a standard lithium solution to each working standard and sample. The optimum lithium concentration may vary among individual instruments; therefore, ascertain it for the instrument used.

Minimise interference by the following:

i. Operate in the lowest practical sodium concentration range.

ii. Add radiation buffers to suppress ionization and anion interference. Among common anions capable of causing radiation interference are Cl^-, $SO4^-$ and HCO_3^- in relatively large amounts.

iii. Introduce identical amounts of the same interfering substances present in the sample into the calibration standards.

iv. Prepare a family of calibration curves embodying added concentrations of a common interference.

v. Apply an experimentally determined correction in those instances where the sample contains a single important interference.

vi. Remove interfering ions.

vii. Remove burner-clogging particulate matter from the sample by filtering through a quantitative filter paper of medium retentiveness.

viii. Incorporate a non-ionic detergent in the standard lithium solution to assure proper aspirator function

ix. Use the standard addition techniques as described in the flame photometric method for strontium. Its use involves adding an identical portion of sample to each standard and determining the sample concentration by mathematical or graphical evaluation of the calibration curve.

x. Use the internal standard technique. Potassium and calcium interfere with sodium determination by the internal standard method, if the potassium to sodium ratio is $\geq$5:1 and the calcium to sodium ratio is $\geq$10:1. When these rations are exceeded, measure calcium and potassium first so that the approximate concentration of interfering ions may be added to the sodium calibration standards. Magnesium interference does not appear until the magnesium to sodium ratio exceeds 100, a rare occurrence.

Minimum detectable concentration: The better flame photometers can be used to determine sodium levels approximating 100µg/L. With proper modifications in techniques the range of sodium measurement can be extended to 10µg/L or lower.

24.3 Apparatus and equipment

i. Flame photometer (either direct-reading or internal-standard type) or atomic absorption spectrometer in the flame emission mode.

24.4 Reagents and standards

.To minimise sodium contamination store all solution in plastic bottles. Use small containers to reduce the amount of dry element that may be picked up from the bottle walls when the solution is poured. Shake each container thoroughly to wash accumulated salt from walls before pouring solution.

i. Deionised distilled water: Use deionised distilled water to prepare all reagents and calibration standards and as dilution water.

ii. Stock sodium solution: Dissolve 2.542 g NaCl dried at 140°C and dilute to 1000mL with water, 1 mL = 1 mg Na.

iii. Intermediate sodium solution: Dilute 10 mL stock sodium solution with water to 100mL; 1 mL = 100µg Na. Use this intermediate solution to prepare calibration curve in sodium range of 1 to 10 mg/L.

iv. Standard sodium solution: Dilute 10 mL intermediate sodium solutions with water to 100 mL; 1.00 mL = 10µg Na. Use this solution to prepare calibration curve in sodium range of 0.1 to 1 mg/L.

24.5 Procedure

Pre-treatment of polluted water and wastewater samples: Filter the sample passing through 0.45µm membrane filter.

Instrument operation: Because of differences between makes and models of instruments, it is impossible to formulate detailed operating instructions. Follow manufacturer's recommendation for selecting proper photocell and wavelength, adjusting slit width and sensitivity, appropriate fuel and air or oxygen pressures and the steps for warm-up, correcting for interferences and flame background, rinsing of burner, igniting sample and measuring emission intensity.

Direct-intensity measurement: Prepare a blank and sodium calibration standards in stepped amounts in any of the following applicable ranges: 0 to 1.0, 0 to 10, or 0 to 100 mg/L. Starting with the highest calibration standard and working toward the most dilute, measure emission

at 589 nm. Repeat the operation with both calibration standards and samples enough times to secure a reliable average reading for each solution. Construct a calibration curve from the sodium standards. Determine sodium concentration of sample from the calibration curve. Where a large number of samples must be run routinely, the calibration curve provides sufficient accuracy.

Internal-standard measurement: To a carefully measured volume of sample (or diluted portion), each sodium calibration standard and a blank, add with a volumetric pipette, an appropriate volume of standard lithium solution. Measure the intensity directly.

Bracketing approach: From the calibration curve, select and prepare sodium standards that immediately bracket the emission intensity of the sample. Determine emission intensities of the bracketing standards (one sodium standard slightly less and the other slightly greater than the sample) and the sample as nearly simultaneously as possible. Repeat the determination on bracketing standards and sample. Calculate the sodium concentration by the equation formed by standard calibration curve.

24.6 Calculation

i. For direct reference to the calibration curve: mg Na/L = (mg Na/L in portion) x D

ii. For the bracketing approach:

$$\text{Mg Na/L} = [\{(B-A)(s-a)+A(b-a)/(b-a)\}]D$$

Where:

B = mg Na/L in upper bracketing standard,

A = mg Na/L in lower bracketing standard,

b = emission intensity of upper bracketing standard,

a = emission intensity of lower bracketing standard,

s = emission intensity of sample and

D = dilution ratio

D = mL sample + mL water /mL sample

24.7 Bibliography

1. Bills, E. et. al., (1949). Reduction of error in flame photometry. Anal. Chem., 21:1076.

2. Gilbert, P.T., R.C. Hawes and A.O. Beckman, (1950). Application of flame spectrophotometry to water analysis. Anal. Chem., 22:667.

3. West, P.W., P. Folse and D. Montgomery, (1950). Application of flames spectrophotometry to water analysis. Anal. Chem., 22:667.

4. American Society For Testing And Materials, (1952). Symposium on flame photometry. Spec. Tech Pub. 116, American Soc. Testing and Materials, Philadelphia, Pa.

5. Collins, E.G. and H. Polkinhorne, (1952). An investigation of anionic interference in the determination of small quantities of potassium and sodium with a new flame photometer. Analyst, 77:430.

6. Dean, J.A., (1960). Flame Photometry, McGraw-Hill Publishing Co., New York, N.Y.

POTASSIUM (K)

25

25.1 Scope and application

Potassium ranks seventh among the elements in order of abundance, yet its concentration in most drinking water seldom reaches 100mg/L. Potassium is an essential element in both plant and human nutrition and occurs in groundwater as a result of mineral dissolution.

Storage of samples: Do not store samples in soft-glass bottles because of the possibility of contamination from leaching of the glass. Use acid washed polyethylene or borosilicate glass bottles. Adjust sample to pH <2 with nitric acid. This will dissolve potassium salts and reduce adsorption on vessel walls.

Methods for analysis

A. Inductively coupled plasma method

B. Flame Emission Photometric method

A. Inductively coupled plasma method

Please refer section 28.6.A.

B. Flame Photometric method

25.2 Summary of the method

Principle: Trace amounts of potassium can be determined in either a direct-reading of internal-standard type of flame photometer at a wavelength of 766.5 nm. Because much of the information pertaining to sodium applies equally to the potassium determination, carefully study the entire discussion dealing with the flame photometric determination of sodium before making a potassium determination.

Interference: Interference in the internal-standard method may occur at sodium-to-potassium ratios of 5:1 or greater. Calcium may interfere if the calcium-to-potassium ratio is 10:1 or more. Magnesium begins to interfere when the magnesium-to-potassium ratio exceeds 100:1.

Minimum detectable concentration: Potassium levels of approximately 0.1 mg/L can be determined.

25.3 Apparatus and equipment

i. Flame photometer: (either direct-reading or internal-standard type) or atomic absorption spectrometer in the flame emission mode.

25.4 Reagents and Standards

To minimise potassium pickup, store all solutions in plastic bottles. Shake each container thoroughly to dissolve accumulated salts from walls before pouring.

i. Reagent water deionised distilled water: Use this water for preparing all reagents and calibration standards and as dilution water.

ii. Stock potassium solution: Dissolve 1.907g KCl dried at 110°C and dilute to 1000mL with water; 1mL = 1mg K.

iii. Intermediate potassium solution: Dilute 10mL stock potassium solution with water to 100mL; 1 mL = 0.1 mg K. Use this solution to prepare calibration curve in potassium of 1 to 10 mg/L.

iv. Standard potassium solution: Dilute 10mL intermediate potassium solution with water to 100mL; 1mL = 0.01 mg K. Use this solution to prepare calibration curve in potassium range of 0.1 to 1 mg/L.

25.5 Procedure

Pre-treatment of polluted water and wastewater samples: Please refer section 28.11.

Instrument operation: Because of differences between makes and models of instruments, it is impossible to formulate detailed operating instructions. Follow manufacturer's recommendation for selecting proper photocell and wavelength, adjusting slit width and sensitivity, appropriate fuel and oxidant gas pressures and the steps for warm-up, correcting for interference and flame background, rinsing of burner, igniting flame and measuring emission intensity.

Direct-intensity measurement: Prepare a blank and potassium calibration standards in stepped amount in any of the following applicable ranges: 0 to 1.0, 0 to 10, 0 to 100 mg/L. Determine emission intensity at 766.5 nm. Aspirate calibration standards and a samples enough time to secure a reliable average reading for each. Construct a calibration curve from the potassium standards. Determine potassium concentration of sample from the calibration curve. Where a large number of samples must be run routinely, the calibration curve provides sufficient accuracy.

25.6 Calculation

For direct reference to the calibration curve:

Mg K/L = (mg K/L in portion) x D

Where, D = Dilution factor

25.7 Bibliography

1. Mehlich, A. and R. J. Monroe, (1952). Report on potassium analyses by means of flame photometer methods, J. Assoc. Ottic. Agr. Chem., 35:588

26

FLUORIDE (F^-)

Introduction:

Fluoride ions have dual significant in water supplies. High concentration of F^- causes dental fluorosis (disfiguerment of the teeth). At the same time, a concentration less than 0.8mg/L results in 'dental caries'. Hence, it is essential to maintain the F^- concentration between 0.8 to 1.0mg/L in drinking water. Among the many methods suggested for the determination fluoride ion in water, the Colorimetric method (SPANDS) and the ion selective electrode method are the most satisfactory and applicable to variety of samples. Because all of the Colorimetric methods are subject to errors due to presence of interfering ions, it may be necessary to distill the sample before making the fluoride estimation.

A. Ion selective electrode method

This method can be used to determine the concentration of ionic species in water and is known as direct potentiometry. The electrode whose potential depends upon the concentration of the ions to be determined is termed as the indicator electrode. The electrodes are used with a reference electrode and for this purpose a silver-silver chloride electrode is usually prepared. A double junction reference electrode is used for measurement of pH. The glass membrane of the electrode when replaced by other materials such as a single crystal or solid ion exchange material, it gives electrode, capable of measuring the concentration available for determination of pH, sodium, potassium, calcium, nitrate, chlorite, silver, lead, cadmium, copper, fluoride, bromide, iodide, cyanide, thiocyanide and Sulphide. The fluoride-sensitive electrode is of the solid state type, consisting of a lanthanum fluoride crystal; in use it forms a cell in combination with a reference electrode, normally the calomel electrode. The crystal contacts the sample solution at one face and an internal reference solution at the other.

26.1 Principle

When the fluoride electrode is dipped in sample whose concentration is to be measured, a potential is established by the presence of fluoride ions by any modern pH meter having an expanded millivolt scale.

The fluoride ion selective electrode can be used to measure the activity or concentration of fluoride in aqueous sample by use of an appropriate calibration curve. However, fluoride activity depends on the total ionic strength of the sample. The electrode does not respond to

bound or complexed fluoride. Addition of a buffer solution of high total ionic strength containing a chelate to complex aluminium preferentiality overcomes these difficulties.

26.2 Apparatus and equipment

a. Ion meter (field/laboratory mode) or pH/mV meter for precision laboratory measurements

b. Reference electrode (calomel electrode)

c. Fluoride-sensitive electrodes

d. Magnetic stirrer

e. Plastic lab ware (samples and standards should always be stored in plastic containers as fluoride reacts with glass).

26.3 Reagents and standards

a. Stock fluoride solution: dissolve 221mg anhydrous NaF and dilute to 1000mL. 1mL = 100µgF$^-$

b. Standard fluoride solution: Dilute stock solution 10 times with distilled water to obtain 1mL = 10µg F$^-$

c. Total Ionic Strength Adjustment Buffer (TISAB): place approximately 500mL distilled water in a 1L beaker, add 57mL glacial acetic acid, 58g, NaCl and 4g 1, 2-cyclohexylenediamine tetraacetic acid. Stir to dissolve. Place beaker in a cool water bath and add slowly 6N NaOH (about 125mL) with stirring, until pH is between 5 to 5.5. Transfer to a 1L volumetric flask and make up the volume to the mark.

26.4 Sample collection, preservation and storage

Polyethylene bottles are preferred for collecting and storing samples for fluoride analysis. Glass bottles are satisfactory, provided that they have not previously contained high-fluoride solutions. Always rinse the bottle with a portion of the sample. Sodium thiosulphate in excess of 100mg/L will interfere by producing a precipitate.

26.5 Calibration

Take 50mL of each 1ppm and 10ppm fluoride standard. Add 50mL TISAB (or 5mL if conc. TISAB is used) and calibrate the instrument.

26.6 Procedure

a. For connecting the electrodes to meter and for further operation of the instrument follow the instruction manual supplied by the manufacturer.

b. Check the electrode slope with the ion meter (59.16mV for mono valent ions and 29.58mV for divalent ions at 25°C).

c. Transfer 50 to 100mL of sample to a 150mL plastic beaker. Add TISAB.

d. Rinse electrode, blot dry and place in the sample. Stir thoroughly and note down the steady reading on the meter.

e. Recalibrate every 1 or 2 hours.

f. Direct measurement is a simple procedure for measuring a large number of samples. The temperature of samples and standard should be the same and the ionic strength of standard and samples should be made the same by addition of TISAB to all solutions.

g. Direct measurement results can be verified by a known addition procedure. The known addition procedure involves adding a standard of known concentration to a sample solution. From the change in electrode potential before and after addition, the original sample concentration is determined.

26.7 Calculation

The concentration in mg/L is obtained directly from the specific ion meter.

26.8 Precision and Bias

When using an expanded-scale pH meter or selective-ion meter, recalibrate the electrode frequently by checking the potential reading of the 1mg/L F standard and adjust the calibration control, if necessary, until the meter reads as before. Confirm the calibration after each unknown and also after reading each standard when preparing the standard curve. Use the recovery of known addition as part of regular analytical protocol.

26.9 Interferences

Polyvalent cations such as AI (III) and Si (IV) will complex fluoride ions. However, the addition of CDTA (cyclohexylene diamine tetra acetic acid) preferentially will complex concentrations of aluminium up to 5mg/L. Hydrogen ion forms a complex with fluoride while hydroxide ion interferes with electrode response. By adjusting the pH between 5 and 8 no interference occurs.

26.10 Pollution prevention and waste management

The chemicals are used in micro quantities. No health-hazardous chemicals are used. There is little need for waste management as no large volumes of solvents or hazardous chemicals are used. The laboratory waste management practices be followed so that the protection of water and land by minimizing and controlling all releases from bench operations will be possible.

B. Sodium 2-(parasulphophenylazo)-1,8-dihydoxy-3,6-naphthalene disulphonate (SPADNS) method

This method is used for estimation of fluoride in natural water in the concentration range 0-1.4 mg/L.

26.11 Principle

Under acidic condition fluoride (F^-) reacts with zirconium-SPANDS- dye-lake, dissociating a portion of it into a colourless complex anion (ZrF_6^-) and the dye. As the amount of fluoride increases, the colour produced becomes progressively lighter and hence it obeys Beer's law in a reverse manner. The chemical reaction involved in the method is given below:

(Zr-SPANDS-lake) + 6 F^- ® (SPANDS Red bleached) + ZrF_6^-

26.12 Apparatus and equipment

a. Distillation apparatus
b. Colorimetric for use at 570nm
c. Nessler tubes cap. 100mL.
d. Pipette

26.13 Reagents and standards

a. Sulphuric acid: H_2SO_4, Conc.
b. Silver sulphate: Ag_2SO_4, crystals.
c. SPANDS solution: dissolve 958 mg SPANDS in distilled water and dilute to 500mL. This solution is stable for atleast 1 year if protected from direct sunlight.
d. Zirconyl acid reagent: dissolve 133mg $ZrOCl_2.8H_2O$ in 25mL water. Add 350mL conc. HCl and dilute to 500mL.
e. Acid Zirconyl-SPANDS reagent: Mix equal volume of 'c' and'd' to produce a single reagent. Protect from direct light. This combined reagent is suitable for atleast 2 years.
f. Reference solution: add 10mL SPANDS solution to 100mL distilled water. Dilute 7mL conc. HCl to 10mL and add to dilute SPANDS solution. Use this solution for setting zero of spectrophotometer. It is stable for 1 year. Alternatively use a prepared standard of 0mg F^-/L as a reference.
g. Sodium arsenite solution: dissolve 5.0g NaAsO2 and dilute to 1000mL. (Caution: Toxic-avoid ingestion)

h. Stock fluoride solution: dissolve 221mg anhydrous NaF and dilute to 1000mL. 1mL = 100µg F^-.

i. Standard fluoride solution: dilute stock solution 10 times with distilled water to obtain 1 mL = 10 µg F^-.

26.14 Sample collection, preservation and storage

Refer section 26.4.

26.15 Calibration

Prepare fluoride standards in the range of 0 to 1.4 mg/L by diluting appropriate quantities of the standard fluoride solution to 50mL with distilled water. Pipette out 5.00mL each of SPANDS solution and Zirconyl-acid reagent, or 10mL of the mixed acid-Zirconyl-SPANDS reagent. Add to each standard and mix well. Avoid contamination during the process. Set the photometer to zero absorbance with the reference solution and obtain the absorbance readings of the standards immediately. Plot a curve of the fluoride-absorbance relationship.

Prepare a new standard curve whenever a fresh reagent is made or a different standard temperature is desired. If no reference solution is used, set the photometer at some convenient point established with a prepared fluoride standard. If the standard contains residual chlorine remove it by addition of $NaAsO_2$.

26.16 Procedure

Preliminary distillation:

Place 400mL distilled water in the distilling flask and carefully add 200 mL conc. H_2SO_4. Swirl until the flask contents are homogeneous, add 25-30 glass beads and connect the apparatus as shown in Fig. 10.1.1. Begin heating slowly at first and then rapidly until the temperature of the flask reaches exactly 180°C. Discard the distillate. This process removes fluoride contamination and adjusts the acid-water ratio for subsequent distillations. After cooling acid mixture remaining after above step or previous distillation to 80°C or below, add 300mL of sample, mix thoroughly, and distill as before until the temperature reaches 180°C. Do not heat above 180°C to prevent sulphate carry-over.

Add Ag_2SO_4 to distilling flask at the rate of 5 mg/mg Cl^- when high chloride samples are distilled.

Use the sulphuric acid solution in the flask repeatedly until the contaminants from the samples accumulate to such an extent that recovery is affected or interferences appear in the distillate. Check the suitability of acid periodically by distilling standard fluoride samples. After distilling of samples containing more than 3mg F^-/L, flush the still with 300mL

distilled water and combine the two fluoride distillates. After periods of inactivity similarly flush the still, discard the distillate.

Colour development

a. Prepare standard curve in the range 0.0 to 1.40 mg/L by diluting appropriate volume of standard fluoride solution to 50mL in Nessler tubes.
b. Add 10.0mL acid Zirconyl-SPANDS reagent to all the samples, mix well and read optical density colour at 570nm using reference solution for setting zero absorbance.
c. Plot concentration vs per cent transmission or absorbance.
d. If the sample contains residual chlorine, remove it by adding drop (0.05mL) $NaAsO_2$ Soln./0.1mg Cl and mix (sodium arsenite concentrations of 1,300 mg/L produce an error of 0.1mg/L at 1.0mg/L F^-).
e. Add 10mL. Acid Zirconyl-SPANDS reagent, mix well and read% transmission or absorbance.
f. Take suitable aliquots of sample either direct or after distillation in Nessler tubes. Follow the step (e).
g. Calculate mg F^-/L present in the sample using standard curve.

26.17 Calculation

$\mu g/L, F = (A / mL\ sample) \times (B/C)$

where A = µg F^- determined photometrically. The ration B/C applies only when a sample is diluted to a volume B, and portion C taken from it for colour development.

26.18 Precision and Bias

Synthetic samples containing fluoride 570-830 µg/L when analysed different laboratories showed relative standard deviation 2.8-8% and relative error 1.22-5.9%. Use the recovery of known addition as part of regular analytical method.

26.19 Interferences

Alkalinity 5000mg/L, aluminium 0.1 mg/L, chlorides 72000mg/L, Fe 10 mg/L, Phosphate 16mg/L, Sulphate 200mg/L, and hexametaphosphate 1.0mg/L interfere in the bleaching action. Residual chlorine interferes in bleaching action. In presence of interfering radicals distillation of sample is recommended.

26.20 Pollution prevention and waste management

Refer section 26.10.

26.21 References

a. Harwood, J.E., (1969). The use of an ion-selective electrode for routine analysis of water samples, Water Res, 3:273.

b. Standard Methods for the Examination of Water and Wastewater; APHA, AWWA and WEF, 21st Edition, 2005.

c. Bellack, E., (1958). Simplified fluoride distillation method. J. American Water works Association, 50:330.

d. Bellack, E. P.J. Schoubof, (1968). Rapid photometric determination of fluoride with SPANDS-zirconium Lake. Anal. Chem., 30:2032.

CHLORIDE (Cl^-)

27

Introduction

The presence of chloride in natural waters can be attributed to dissolution of salt deposits, discharges of effluents from chemical industries, oil well operations and seawater intrusion in coastal areas. Each of these sources may result in local contamination of both surface water and groundwater. The salty taste produced by chloride depends on the chemical composition of the water. A concentration of 250mg/L may be detectable in some waters containing sodium ions. On the other hand, the typical salty taste may be absent in water containing1000mg/L chloride when calcium and magnesium ions are predominant. High chloride content may harm pipes and structures as well as agricultural plants.

A. Argentometric method

This method is used for the analysis of the chloride ion present in the natural water. The mercurimetric method is recommended when an accurate determination of chloride is required, particularly at low concentrations. The potentiometric method is suitable only when the sample is coloured or turbid, argentometric method is the simplest one can be the method of choice for variety of samples.

27.1 Principle

The quality of sample for estimation of chloride should be 100mL or a suitable portion diluted to 100mL. Chloride is determined in a natural or slightly alkaline solution by titration with standard silver nitrate, using potassium chromate as indicator.

Silver chloride is quantitatively before red silver chromate is formed. The chemical reactions involved in this method are given below:

1. $Ag^{++} Cl^- \rightarrow AgCl$ (White precipitate)
2. $2Ag^{++} CrO_4^{--} \rightarrow Ag_2CrO_4$ (Red precipitate)

27.2 Apparatus

a. Porcelain dish, 200mL

b. Pipettes

c. Burettes

d. Glass rod

27.3 Reagents and standards

a. Potassium chromate indicator: dissolve 50g $K_2Cr_2O_7$ in distilled water. Add $AgNO_3$ till definite red precipitate is formed. Allow to stand for 12hrs. Filter and dilute to 1000mL.

b. Silver nitrate, 0.0141N: Dissolve 2.395g $AgNO_3$ and dilute to 1000mL. Standardise against NaCl, 0.0141N; 1mL of 0.0141N $AgNO_3$ = 0.5 mg Cl^-.

c. Sodium chloride, 0.0141N: dissolve 824.1mg NaCl (dried at 40°C) and dilute to 1000mL; 1mL = 0.5 mg Cl^-.

d. Special reagent to remove colour and turbidity: dissolve 125g $AlK(SO_4)_2.12H_2O$ or $AlNH_4(SO_4)_2.12H_2O$ and dilute to 1000mL. Warm to 60°C and add 55mL conc. NH_4OH slowly. Let stand for 1 hour. Transfer to a large bottle and wash precipitate by successive addition with thorough mixing and decanting with distilled water until free from chloride. When freshly prepared, a suspension occupies a volume of approximately 1L.

27.4 Calibration

The silver nitrate solution should be standardised against sodium chloride solution of 0.0141N. It gives the strength of silver nitrate solution 1mL = 0.5mg chlorides as Cl^-.

27.5 Procedure

a. Take 50mL well mixed sample adjusted to pH 7.0-8.0and add 1.0 mL $K_2Cr_2O_7$

b. Titrate with standard $AgNO_3$ solution till $AgCrO_4$ starts precipitating as pale red precipitate

c. Standardise $AgNO_3$ against standard NaCl

d. For better accuracy titrate distilled water (50mL) in the same way to establish reagent blank. A blank of 0.2 to 0.3mL is usual.

27.6 Calculation

chloride mg/L as Cl^- = (A B) x N x 35.45 x 1000 / mL sample

where,

A = mL $AgNO_3$ required for sample

B = mL $AgNO_3$ required for blank, and

N = Normality of $AgNO_3$ used

27.7 Precision and Bias

A synthetic unknown sample containing 241mg/L chloride, 105mg/L Ca, 82mg/L Mg, 3.1mg/LK, 19.9 mg/L Na, 1.1mg/L nitrate, 0.25mg/L nitrite N, 259mg/L sulphate, and 42.5mg/L total alkalinity (contributed by $NaHCO_3$) in distilled water was analysed in 41 laboratories by the argentometric method, with a relative standard deviation of 4.2% and a relative error of 1.7%.

For quality assurance run the sample in duplicate. Individual blank may differ from person to person; so it is necessary to carry out blank by using distilled water as sample and apply the same procedure as sample for blank during analysis.

27.8 Interferences

Bromide, iodide and cyanide are measured as equivalent of chloride ions, if the sample contains sufficient thiosulphate, thiocyanate, cyanide, sulphate and sulphide to interfere seriously with the determination. If the sample is too coloured or turbid to allow the end point to be readily detected, this interference may be reduced by the following treatment with a suspension of aluminium hydroxide. Add 3mL aluminium hydroxide suspension to the measured quantity of sample. Stir thoroughly, set aside for a few minutes and filter. Wash the precipitate with distilled water. Collect the washings with the filtrate and continue as described in procedure.

27.9 Pollution prevention and waste management

There is little need for waste management as no large volumes of solvents or hazardous chemicals are used. However, the chemicals required for the analysis are indicated in a separate chapter on waste management. The laboratory waste management practices be followed so that the protection of water and land by minimizing and controlling all releases from bench operations will be possible.

27.10 Reference

1. Standard Methods for the Examination of Water and wastewater; APHA, AWWA and WEF, 21st Edition, 2005.

28.1 Aluminium (Al)

28.1.1 Scope and application

Aluminium is the third most abundant element on the earth's crust. The presence of aluminium in all natural water is in the form of soluble salt, a colloid, or an insoluble compound. Soluble, colloidal, and insoluble aluminium may be present in treated water in residual form of coagulation with aluminium-containing material. The USEPA water standard for Al is 0.05mg/L max. BIS desirable limit is 0.03mg Al/L.

Methods for analysis

A. Inductively coupled plasma/ AAS

B. Eriochrome cyanine R method

A. Inductively coupled plasma method

Please refer section 28.6A.

B. Eriochrome cyanine R method

28.1.2 Summary of method

Principle:

With Eriochrome Cyanine R dye, dilute aluminium solutions buffered to a pH 6.0 produce a red to pink complex that exhibits maximum absorption at 535 nm. The aluminium concentration, reaction time, temperature, pH, alkalinity, and concentration of other ions in the sample influence the intensity of the complex. To compensate for colour and turbidity, the aluminium is one portion of sample is complexed with EDTA to provide a blank. Adding ascorbic acid eliminates the interference of iron and manganese. The optimum range lies between 20 and 30µg/L but can be extracted by sample dilution.

Interference:

Both fluoride and polyphosphates cause negative errors. When the fluoride concentration is constant, the percentage error decrease with increasing amount of aluminium. Because the

fluoride concentration often is known or can be determined readily, fairly accurate results can be obtained by adding the known amount of fluoride to a set of standards. Orthophosphate in concentrations under 10mg/L does not interfere. The interference caused by even small amount of alkalinity is removed by acidifying the sample just beyond the neutralization point of methyl orange. Sulphate does not interfere up to a concentration of 2000mg/L.

Minimum detectable concentration:

The minimum aluminium concentration detectable by this method in the absence of fluorides and complex phosphates is approximately 6µg/L.

28.1.3 Apparatus and equipment

i. Spectrophotometer, wavelength at 535nm, with a light path of 1cm.

ii. Glassware: Treat all glassware with warm 1+1 HCl and rinse with aluminium free distilled water to avoid errors due to materials absorbed on the glass. Rinse sufficiently to remove all acid.

28.1.4 Reagents and standards

Use reagents and distilled water free from aluminium contamination.

i. Stock aluminium solution: Use either the metal (1) or soluble salt (2) for preparing stock solution; 1mL = 500µg Al: 1) Dissolve 500mg aluminium metal in 10mL conc. HCl by heating gently. Dilute to 1000mL with distilled water, 2) Dissolve 8.791g aluminium potassium sulfate, AlK $(SO_4)_2$. $12H_2O$, in water and dilute to 1000mL. Correct this weight by dividing by the decimal fraction of analysed AlK $(SO_4)_2$.$12H_2O$ in the reagent used.

ii. Standard aluminium solution: Dilute 10.0mL stock aluminium to 1000mL with water; 1mL = 5.00µg Al. Prepare fresh stock.

iii. Sulphuric acid, H_2SO_4, 0.02N and 6N.

iv. Ascorbic acid solution: Dissolve 0.1-g ascorbic acid in water and make up to 100mL in volumetric flask. Prepare fresh daily.

v. Buffer reagent: Dissolve 136g sodium acetate, $NaC_2H_3O_2.3H_2O$, in water, add 40mL 1N acetic acid, and dilute to 1L.

vi. Stock dye solution: Eriochrome cyanine: R: Dissolve 100mg in water and dilute to 100mL in volumetric flask. This solution should have a pH of about 9 to 2.9. Stock solutions have excellent stability and can be kept for at least a year.

vii. Working dye solution: Dilute 10mL of selected stock dye solution to 100mL in volumetric flask with water. Working solutions are stable for at least 6 months.

viii. Methyl orange indicator solution (or) bromocresol green indicator solution: as in alkalinity test

ix. EDTA (sodium salt of ethylenediamine-tetraacetic acid dehydrate): 0.01M: Dissolve 3.7g in water, and dilute to 1L.

x. Sodium hydroxide: NaOH, 1N and 0.1N.

28.1.5 Procedure

Preparation of calibration curve: Prepare a series of aluminium standards from 0 to 7µg (0 to 280µg/L) based on a 25mL sample by accurately measuring the calculated volumes of standard aluminium solution into 50mL of volumetric flasks or Nessler tubes. Add water to a total volume of approximately 25mL.

Add 1mL of 0.02N H_2SO_4 to each standard and mix. Add 1mL ascorbic acid solution and mix. Add 10mL buffer solution and mix. With a volumetric pipette, add 5.00mL working dye reagent and mix. Immediately make up to 50mL with distilled water. Mix and let it stand for 5 to 10 min. The colour begins to fade after 15min. Read transmittance or absorbance on a spectrophotometer, using a wavelength of 535nm. Adjust instruments to zero absorbance with the standard containing no aluminium. Plot concentration of Al (micrograms Al in 50mL final volume) against absorbance.

Sample treatment in absence of fluorides and complex phosphate:

Place 25mL sample, or a portion diluted to 25mL, in a porcelain dish or flask, add a few drops of methyl orange indicator, and titrate with 0.02N H_2SO_4 to faint pink colour. Record reading and discard sample. To two similar samples at room temperature add the same amount of 0.02N H_2SO_4 used in the titration and 1mL in excess.

To one sample add 1mL EDTA solution. This will serve as a blank by complexing any aluminium present and compensating for colour and turbidity. To both samples add 1mL ascorbic acid, 10mL buffer reagent and 5mL working dye reagent.

Set instrument to zero absorbance or 100% transmittance using the EDTA blank. After 5 to 10 minutes contact time, read transmittance or absorbance and determine aluminium concentration from the calibration curve previously prepared.

Removal of phosphate interference:

Add 1.7mL 6H2SO4 to 100mL sample in a 200mL Erlenmeyer flask. Heat on a hot plate for at least 90 min, keeping solution temperature just below the boiling point. At the end of the heating period the solution volume should be about 25mL. Add water if necessary to keep it at or above that volume.

After cooling, neutralize to a pH of 4.3 to 4.5 with NaOH, using 1N NaOH at the start and

0.1N for the final time adjustment. Monitor with a pH meter. Make up to 100mL with water, mix and use a 25mL portion for the aluminium test.

Run a blank in the same manner, using 100mL distilled water and 1.7mL $6H_2SO_4$. Subtract blank reading from sample reading or use it to set instrument to zero absorbance before reading the sample.

Correction for samples containing fluoride

Add the same amount of fluoride as in the sample to each aluminium standard, and draw calibration curve for aluminium standard

28.1.6 Calculation

MgAl/L = µg Al (in 50mL final volume) / mL sample

28.1.7 Bibliography

1. Shull, K.E. and G.R. Guthan, I., (1967). Rapid modified Eriochrome Cyanine R method for determination of aluminium in water. J. Amer. Water Works Assoc., 59:1456.

28.2 Arsenic (As)

28.2.1 Scope and application

Severe poisoning can arise from the ingestion of as little as 100mg of Arsenic trioxide. Chronic effects may result from the accumulation of arsenic compounds in the body at low intake levels. Carcinogenic properties also have been observed to arsenic compounds. For the protection of aquatic life, the average concentration of As^{3+} in water should not exceed 72µg/L. And maximum should not exceed 140µg/L. FAO-recommended max level for irrigation water is 100µg/L. USEPA water standard is 0.05mg/L. BIS desirable limit is 0.05mg As/L.

Methods for analysis:

A. Silver diethyldithiocarbamate method

B. Inductively coupled plasma method

A. Silver diethyldithiocarbamate method

28.2.2 Summary of method

Principle: Arsenite, containing trivalent arsenic, is reduced selectively by aqueous sodium borohydride solution to arsine, AsH_3, in an aqueous medium of pH 6. Arsenate, methyl arsenic acid, and dimethyl arsenic acid are not reduced under these conditions. The generated arsine is swept by a stream of oxygen-free nitrogen from the reduction vessel through a scrubber containing glass wool or cotton impregnated with lead acetate solution into an absorber tube containing silver diethyldithiocarbamate and morpholine dissolved in chloroform. A red colour intensity that develops is measured at 520nm. To determine total inorganic arsenic in the absence of methyl arsenic compounds, reduce another sample portion at a pH of about 1.0. Alternatively, determine arsenate in a sample from which arsenite has been removed by reduction at pH 6. The sample is acidified with hydrochloric acid and another portion of sodium borohydride solution is added. Arsine formed from arsenate is collected in fresh absorber solution.

Interferences: Although certain metals, viz. chromium, cobalt, copper, mercury, molybdenum, nickel, platinum, silver, and selenium influence the generation of arsine, their concentrations in water seldom are high enough to interfere. H_2S interferes, but the

interference is removed with lead acetate. Antimony is reduced to stibine, which forms a coloured complex with absorption maximum at 510nm and interferes with the arsenic determination. Methylarsenic compounds are reduced at pH 1to methylarsines, which form coloured complexes with the absorber solution. If methylarsenic compounds are present, measurements of total arsenic and arsenate are unreliable. Methyl arsenic compounds do not influence the results for arsenite.

Minimum detectable quantity: 1µg arsenic.

28.2.3 Apparatus and equipment

Arsine generator, scrubber, and absorption tube:

Use a 200mL three-necked flask with a sidearm (19/22 or similar size female ground-glass joint) through which the inert gas delivery tube reaching almost to the bottom of the flask is inserted; a 24/40 female ground-glass joint to carry the scrubber; and a second side arm closed with a rubber septum, or preferably by a screw cap with a hole in its tip for inspection of a TFE-faced silicon septum. Place a small magnetic stirring bar in the flask. Fit absorber tube (20mL capacity) to the scrubber and fill with silver diethyldithiocarbamate solution. Do not use rubber or cork stoppers because they may absorb arsine. Clean glass equipment with concentrated nitric acid.

Fume hood: Use apparatus in a well-ventilated hood with flask secured on top of a magnetic stirrer.

Spectrophotometer: for use at 520nm with current 1cm path.

28.2.4 Reagents and standards

Use reagents and distilled water free from arsenic contaminations.

i. Acetate buffer: pH 5.5. Mix 428mL 0.2M sodium acetate, $NaC_2H_3O_2$ and 72mL of 0.2mL/L acetic acid, CH_3COOH.

ii. Sodium acetate, 0.2M: Dissolve 16.46g anhydrous sodium acetate, or 27.36g sodium acetate trihydrate, $NaC_2H_3O_2.3H_2O$ in water. Dilute to 1000mL with water.

iii. Acetic acid, 0.2M: Dissolve 11.5mL glacial acetic acid in water. Dilute to 1000mL.

iv. Sodium borohydride solution, 1%: Dissolve 0.4g sodium hydroxide, NaOH (4 Pellets), in 400mL water. Add 4.0g sodium borohydride, $NaBH_4$ (check for absence of arsenic). Shake to dissolve and to mix.

v. Hydrochloric acid (HCl), 2M: Dilute 165mL conc. HCl to 1000mL with water.

vi. Lead acetate solution: Dissolve 10.0g $Pb(CH_3COOH)_2.3H_2O$ in 100mL water.

vii. Silver diethyldithiocarbamate solution: Dissolve 1.0mL morpholine (Caution: Corrosive-avoid contact with skin) in 70mL chloroform, $CHCl_3$. Add 0.30g silver deithyldithiocarbamate, $AgSCSN(C_2H_5)_2$ shake in a stoppered flask until most is dissolved. Dilute to 100mL with chloroform. Filter and store in a tightly closed brown bottle in a refrigerator.

viii. Stock arsenite solution: Dissolve 0.1734g $NaAsO_2$ in water and dilute to 1000mL with water; 1.00mL = 100µg As.

Caution: Toxic-avoid contact with skin and do not ingest.

ix Intermediate arsenite solution: Dilute 10.0mL stock solution to 100mL with water; 1.00mL = 1.00µg As.

x Standard arsenite solution: Dilute 10.0mL intermediate solution to 100mL with water; 1.00 mL = 1.00µg As.

xi Standard arsenate solution: Dissolve 0.416g $Na_2HAsO_4.7H_2O$ in water and dilute to 1000mL. Dilute 10.0mL to 100mL with water; dilute 10mL of this intermediate solution to 100mL; 1.00mL = 1.00µg As.

28.2.5 Procedure

Arsenite: Preparation of scrubber and absorber-Dip glass wool into lead acetate solution; remove excess by squeezing glass wool. Press glass wool between pieces of filter paper, and then fluff it. Alternatively, if cotton is used, treat it similarly but dry in a desiccator and fluff thoroughly when dry. Place a plug of loose glass wool or cotton in scrubber tube. Add 4.00mL silver diethyldithiocarbamate solution to absorber tube (5.00mL may be used to provide enough volume to rinse spectrophotometer cell).

Loading of arsine generator-Pipet not more than 70mL sample containing not more than 20.0µg As (arsenite) into the generator flask. Add 10mL acetate buffer. If necessary, adjust total volume of liquid to 80mL. Flush flask with nitrogen at the rate of 60mL/min.

Arsine generation and measurement-While nitrogen is passing through the system, use a 30mL syringe to inject through the septum 15mL 1% sodium borohydride solution within 2 min. Stir vigorously with magnetic stirrer. Pass nitrogen through system for an additional 15 min to flush arsine into absorber solution. Pour absorber solution into a clean and dry spectrophotometric cell and measure absorbance at 520nm against chloroform. Determination concentration from a calibration curve obtained with arsenite standards. If arsenate also is to be determined in the same sample portion, save liquid in the generator flask.

Preparation of standard curves: Treat standard arsenite sodium containing 0.0, 1.0, 2.0, 5.0, 10.0 and 20.0 µg as described above. Plot absorbance versus micrograms arsenic in the standard.

Arsenate: After removal of arsenite as arsine, treat sample to convert arsenate to arsine. If the lead acetate-impregnated glass wool has become ineffective in removing hydrogen sulphide (if it has become grey to black) replace glass wool. Pass nitrogen through system at the rate of mL/min. Cautiously add mL 2N HCl. Generate arsine with all precautions and prepare standard curves with standard solutions of arsenate.

Total inorganic arsenic: Prepare scrubber and absorber for arsine. Load arsine generator using mL of 2N HCl instead of acetate buffer. Generate arsine and measure the absorbance as directed above. Prepare standard curves. Curves obtained with standard arsenite are almost identical to those obtained with arsenate standard solutions. Therefore, use either arsenite or arsenate standards.

28.2.6 Calculation

Calculate arsenite, arsenate and total inorganic arsenic from readings and calibration curves obtained in 4a, b, anlibration curve) / mL sample in generator flask

28.2.7 Bibliography

1. Aggett, J. and A.C. Aspell, (1976). Determination of arsenic (III) and total arsenic by the silver diethyldithiocarbamate method.
2. Howard, A.G. and M. H. Arbab-Zavar, (1980). Sequential spectrophotometer determination of arsenic (II) and arsenic (V) species. Analyst, 105:338.
3. Pande, S. P., (1980). Morpholine as a substitute for pyridine in determination of arsenic in water. J. Inst. Chem, (India), 52:256.
4. Irgolic, K.J., (1987). Analytical procedures for the determination of organic compounds of metals metalloids in environmental samples. Sci. Total Environment, 64:61.

B. Inductively coupled plasma method

Please refer section 28.6 A.

28.3 Cadmium (Cd)

28.3.1 Scope and application

Cadmium occurs in sulphide minerals that also contain zinc, lead or copper. The metal is used in electroplating, batteries, paint pigments and in alloys with various other metals. Cadmium is usually associated with zinc. Cadmium is highly toxic and has been implicated in some cases of poisoning through food. Minute quantities of cadmium are suspected of being responsible for adverse changes in arteries of human kidneys. Cadmium also causes generally cancers in laboratory animals and has been linked epidemiologically with certain human cancers. A cadmium concentration of 200µg/L is toxic to certain fishes. Cadmium may enter water as a result of industrial discharges or the deterioration of galvanized pipe. The FAO-recommended maximum level for cadmium for irrigation water is 10µg/L/ USEPA drinking water standard for cadmium is 0.005mg/L. BIS desirable limit is 1mg/L.

Methods for analysis:

A. Inductively coupled plasma method/AAS

B. Dithizone method

A. Inductively coupled plasma method

Please refer section 28.6A.

B. Dithizone method

28.3.2 Summary of method

Principle: Cadmium ions under suitable conditions react with dithizone to form a pink to red colour that can be extracted with chloroform ($CHCl_3$). Chloroform extracts are measured photometrically and the cadmium concentration is obtained from a calibration curve prepared from a standard cadmium solution treated in the same manner as the sample.

Interference: Under the specified conditions, concentrations of metal ions normally found in water do not interfere. Lead up to 6mg, zinc up to 3mg, and copper up to 1mg in the portion analysed do not interfere. Ordinary room lighting (incandescent or fluorescent) does not affect the cadmium dithizonate colour.

Minimum detectable concentration: 0.5µg/L Cd with 1cm light path.

28.3.3 Apparatus

i. Spectrophotometer: Use at 518nm with minimum light path of 1cm.

ii. Separatory funnels: 125mL, preferably with TFE stopcocks.

iii. Glassware: Clean all glassware, including sample bottles, with 1+1 HCl and rinse thoroughly with tap water and distilled water.

28.3.4 Reagents and standards

Use reagents and distilled water free from cadmium contamination.

i. Stock cadmium solution: Weigh 100mg pure Cd metal and dissolve in a solution composed of 20mL water plus 5mL conc. HCl. Use heat to assist metal dissolution. Transfer quantitatively to a 1L volumetric flask and dilute to 1000mL; 1.00mL µg Cd. Store in a polyethylene container.

ii. Standard cadmium solution: Pipette 1mL stock cadmium solution into a 100mL volumetric flask, add 1mL conc. HCl, and dilute to 100mL with water. Prepare as required and use on the same day; 1mL = 1µg Cd.

iii. Sodium potassium tartarate solution: Dissolve 250g, $NaKC_4H_4.4H_2O$ in water and make up to 1L.

iv. Sodium hydroxide-potassium cyanide solutions: Solution l: Dissolve 400g NaOH and 10g KCN in water and make up to 1L. Store in a polyethylene bottle. This solution is stable for 1 month.

Solution ll: Dissolve 400g NaOH and 0.5g KCN in water and make up to 1L. Store in a polyethylene bottle. This solution is stable for 1 to 2 months.

Caution: Potassium cyanide is extremely poisonous. Be especially cautious when handling it. Never use mouth pipettes to deliver cyanide solutions.

v. Hydroxylamine hydrochloride solution: Dissolve 20g, NH2OH.HCl in water and make up to 100mL.

vi. Stock dithizone solution: In fume hood, dissolve 100mg dithizone in 50mL $CHCl_3$ in a 150mL beaker and filter through a 7cm diameter paper. Receive filtrate in a 500mL separatory funnel. Wash the paper with three 5mL portion of CHCl3. Transfer with $CHCl_3$ to 500mL separatory funnel. Add 100mL (1+99) NH_4OH to a separatory funnel and shake moderately for 1 min. Let layers separate. Transfer $CHCl_3$ layer to a 250mL separatory funnel retaining the orange red aqueous layer in the 500mL funnel. Repeat extraction receiving $CHCl_3$ layer in another 2520mL NH_4OH, to the 500mL funnel holding the first extract. Repeat extraction

transferring the aqueous layer to 500mL funnel. Discard $CHCl_3$ layer. To combine extracts in the 500mL separatory funnel add (1+1) HCl in 2mL portions mixing after each addition, until dithizone precipitates and solution is no longer red. Extract precipitated dithizone with three 25mL portions $CHCl_3$. Dilute combined extracts to 1000mL with $CHCl_3$; 1mL = 100mg.

vii. Working dithizone solution: Dilute stock dithizone solution with $CHCl_3$ to produce a working solution of 10µg/mL. Prepare daily.

viii. Chloroform: Test for a satisfactory $CHCl_3$ by adding a minute amount of dithizone to a portion of the $CHCl_3$ in a stoppered test tube so that a faint green colour is produced; the green colour should be stable for a day.

ix. Tartaric acid solution: Dissolve 20g, $H_2C_4H_4O_6$ in water and make up to 1L. Store in refrigerator and use while still cold.

x. Hydrochloric acid: conc. HCl

xi. Thymol blue indicator solution: Dissolve 0.4g thymol-sulphonephtalein sodium salt in 100mL water.

xii. Sodium hydroxide: NaOH, 6N.

28.3.5 Procedure

Preparation of standard curve: Prepare a blank and a series of standards by pipetting the appropriate amounts of standard Cd solution into separatory funnels. Dilute to 25mL, and pipette 1mL stock cadmium solution into a 100-mL volumetric flask, add 1mL conc. HCl, and dilute to 100mL with water. Prepare as needed and use the same day; 1mL = 1µg Cd. Plot a calibration curve.

Treatment of samples: Pipette a volume of digested sample containing 1 to 101µg Cd to a separatory funnel and dilute to 25mL as necessary. Add 3 drops thymol blue and adjust with 6N NaOH to the first permanent yellow colour, pH 2.8. Colour development, extraction, and measurement: Add reagents in the following order, mixing after each addition: 1mL sodium potassium tartarate solution, 5mL NaOH-KCN solution, 1mL $NH_2OH.HCl$ solution, and 15mL stock dithizone solution. Stopper the funnels the stopper rather than the stopcock. Drain $CHCl_3$ layer into a second funnel containing 25mL cold tartaric acid solution.

Add 10mL $CHCl_3$ to first funnel; shake for 1min and drain into second funnel. Do not permit aqueous layer to enter second funnel. Because time of contact of $CHCl_3$ with the storing alkali must be kept to a minimum, make the two extractions immediately after adding dithizone (cadmium dithizonate decomposes on prolonged contact with strong alkali saturated with $CHCl_3$).

Shake second funnel for 2min and discard $CHCl_3$ layer. Add 5mL $CHCl_3$, shake 1min, and discard $CHCl_3$ layer, making as close a separation as possible. In the following order, add

0.25mL $NH_2OH.HCl$ solution and 15.0mL working dithizone solution. Add 5mL NaOH-KCN solution II; immediately shake for 1 min and transfer $CHCl_3$ layer into a dry cuvette. Read absorbance at 518 nm against the blank. Obtain Cd concentration from the calibration curve.

28.3.6 Calculation

mg Cd/L = µg Cd (in approx, 15mL final volume) / mL sample

28.3.7 Bibliography

1. Saltzman, B.E, (1953). Colorimetric micro determination of cadmium with dithizone. Anal. Chem., 25:493.
2. Ganotes, J., E. Larson and R. Navone, (1962). Suggested dithizone method for cadmium determination. J. Amer. Water Works Assoc., 54:852.

28.4 Chromium (Cr)

28.4.1 Scope and application

Chromium is found chiefly in chome-iron ore. Chromium is used in alloys, in electroplating and in pigment. Chromium salts are used extensively in industrial processes and may enter a water supply through the discharge of wastes. Chromate compounds frequently are added to cooling water for corrosion control. Chromium may exist in water supplies in both the hexavalent and the trivalents state although the trivalent form rarely occurs in potable water. FAO recommended the maximum limit for irrigation water as 100µg/L. USEPA drinking water standard for chromium is 0.1mg/L.

Methods for analysis:

A. Inductively coupled plasma method/AAS

B. Colorimetric method

A. Inductively coupled plasma method

Please refer section 28.6 A.

B. Colorimetric method

28.4.2 Summary of the method

Principle: This procedure measures only hexavalent chromium (Cr^{6+}). Therefore, to determine total chromium convert all chromium to the hexavalent state by oxidation with potassium permanganate. The hexavalent chromium is determined colorimetrically by reaction with diphenylcarbazide in acid solution. A red-violet colour of unknown composition is produced which is measured at 540 nm. To determine total chromium, digest the sample with a sulphuric-nitric acid mixture and then oxidise with potassium permanganate before reacting with the diphenylcarbazide.

Interferences: The reaction with diphenylcarbazide is nearly specific for chromium. Hexavalent molybdenum and mercury salts will react to form colour with the reagent but the intensities are much lower than that for chromium at specified pH. Vanadium interferes

strongly but concentrations up to 10 times that of chromium will not cause trouble. Iron in concentrations grater than 1mg/L may produce a yellow colour but the ferric ion (Fe^{3+}) colour is not strong and no difficulty is encountered normally.

28.4.3 Apparatus and equipment

i. Spectrophotometer: for use at 540 nm, with a light path of 1cm.

28.4.4 Reagents and standards

Use reagents and distilled water free from Chromium contamination.

i. Stock chromium solution: Dissolve 141.4 mg $K_2Cr_2O_7$ in water and dilute to 100mL; 1mL = 500µg Cr.

ii. Standard chromium solution: Dilute 1mL stock chromium solution to 100mL; 1mL = 5µg Cr.

iii. Nitric acid: HNO_3, conc.

iv. Sulphuric acid: H_2SO_4, conc.; H_2SO_4, 1+1 (18N); H_2SO_4 (6N).

v. Sulphuric acid: H_2SO_4, 0.2N: Dilute 17mL 6N H_2SO_4 to 500mL with water.

vi. Phosphoric acid: H_3PO_4, conc.

vii. Diphenylcarbazide solution: Dissolve 250mg 1,5-diphenylcarbazide (1,5-dipheny-lcarbohydrazide) in 50 mL acetone. Store in a brown bottle. Discard when solution becomes discoloured.

viii. Sodium hydroxide 1N: Dissolve 40g NaOH 1L water. Store in plastic bottle.

28.4.5 Procedure

Preparation of calibration curve:

To compensate for possible slight losses of chromium during analytical operations, treat chromium standards by the same procedure as the sample. Accordingly, pipette measured volumes of standard chromium solution (5µg/L) ranging from 2 to 20mL, to give standards for 10 to 100 µg Cr, into 250mL beakers or conical flasks.

Colour development and measurements:

Add 0.25mL (5 drops) H_3PO_4. Use 0.2N H_2SO_4 and pH meter to adjust solution to pH ± 0.5. Transfer solution to a 100mL volumetric flask, dilute to 100mL and mix. Add 2mL diphenyl carbazide solution, mix and let stand 5 to 10 min for full colour development. Transfer an appropriate portion to 1 cm absorption cell and measure its absorbance at 540nm, using

reagent water as reference. Correct absorbance of a blank carried through the method. From the corrected absorbance, determine micrograms chromium present by reference to the calibration curve.

Construct a calibration curve by plotting corrected absorbance values against micrograms chromium in 102mL final volume. Repeat the experiment with samples.

28.4.6 Calculation

mg Cr/L = [mg Cr (in 102mL final volume) / A] x 100

Where:

A = mL original sample

28.4.7 Bibliography

1. Saltzman, B.E., (1952). Micro determination of chromium with Diphenylcarbazide by permanganate oxidation. Anal. Chem. 24:1016.
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Copper (Cu)

28.5

28.5.1 Scope and application

Copper occurs in nature in its native state, but also found in many minerals, the most important of which are those containing sulphide compounds, but also those with oxides or carbonates. Copper salts are used in water supply systems to control biological growth of reservoirs and distribution pipes and to catalyse in oxidation of manganese. Corrosion of copper-containing alloys in pipe fitting may introduce measurable amounts of copper into the water in a pipe system. FAO recommended maximum level for irrigation water is 200µg/L. US EPA drinking water standard for chromium and copper is 1 mg/L. BIS limit for copper is 0.05 mg/L.

Method for analysis:

A. Inductively coupled plasma (ICP) method/AAS

B. Neocuproine Method

Sampling and storage

Copper ion tends to be absorbed on the surface of sample containers. Therefore, analyse samples as soon as possible after collection. If storage is necessary, use 0.5 mL 1+1 HCl/100 mL samples, or acidify to pH 2 with HNO_3, to prevent this adsorption.

A. Inductively coupled plasma method

Please refer section 28.6 A.

B. Neocuproine method

28.5.2 Summary of method

Principle: Cuprous ion (u^+) in neutral or slightly acidic solution reacts with 2, 9-dimethyl-1, 10-phenanthroline (neocuproine) to form a complex in which 2 moles of neocuproine are bound by 1 mole of Cu^+ ion. The complex can be extracted by a number of organic solvents, including a chloroform-methanol ($CHCl_3$-CH_3OH) mixture, to give a yellow solution. The

reaction is virtually specific for copper; the colour follow Beer's law up to a concentration of 0.2 mg Cu/25mL solvent; full colour development is obtained when the pH of the aqueous solution is between 3 and 9; the colour is stable in $CHCl_3$-CH_3OH for several days.

The sample is treated with hydroxylamine-hydrochloride to reduce cupric ions to cuprous ions. Sodium citrate is used to complex metallic ions might precipitate when the pH is raised. The pH is adjusted to 4 to 6 with NH_4OH, a solution of neocuproine in methanol is added, and the resultant complex is extracted into $CHCl_3$ to an extract volume with CH_3OH, the absorbance of the solution is measured at 457nm.
Interference: Large amounts of chromium and tin may interfere. Avoid interference from chromium by adding sulfurous acid to reduce chromate and complex chromic ion. In the presence of tin or excessive amounts of other oxidising ions, use up to 20mL additional hydroxylamine-hydrochloride solution. Cyanide, sulphide, and organic matter interfere but can be removed by a digestion.

Minimum detectable concentration: The minimum detectable concentration, corresponding to 0.01absorbance, or 98% transmittance, is 3µg Cu when 1cm cell is used and 0.6µg Cu/L when a 5cm cell is used.

28.5.3 Apparatus and equipment

i. Spectrophotometer: Use at 457nm, providing a light path of 1cm cuvette.

ii. Separatory funnels: 125mL, Squibb form, with glass or TFE stopcock and stopper.

28.5.4 Reagents and standards

Use reagent and distilled water free from copper contamination.

i. Stock copper solution: To 200mg polished electrolytic copper wire or foil in a 250mL conical flask, add 10mL water and 5mL conc. HNO_3. After the reaction has slowed, warm gently to complete dissolution of the copper and boil to expel oxides of nitrogen, using precautions to avid loss of copper. Cool, add about 50mL water, transfer quantitatively to a 1 L volumetric flask, and dilute to the mark with water; 1mL = 200 µg Cu.

ii. Standard copper solution: Dilute 50mL stock copper solution to 500mL with water; 1mL = 20µg Cu.

iii. Sulphuric acid: H_2SO_4, conc.

iv. Hydroxylamine-hydrochloride solution: Dissolve 50g $NH_2OH.HCl$ in 450 mL water.

v. Sodium citrate solution: Dissolve 150g $Na_3C_6H_5O_7.2H_2O$ in 400mL water. Add 5mL $NH_2OH.HCl$ solution and 10mL neocuproine reagent. Extract with 50mL $CHCl_3$ to remove copper impurities and discard $CHCl_3$ layer.

vi. Ammonium hydroxide, NH_4OH, 5N: Dilute 330mL conc. NH_4OH (28-29%) to 1000mL with water. Store in a polyethylene bottle.

vii. pH paper: range of 2-10 pH.

viii. Neocuproine reagent: Dissolve 100mg 2, 9-dimethyl-1, 10-phenanthroline hemihydrate in 100mL methanol. This solution is stable under ordinary storage conditions for a month or more.

ix. Chloroform, $CHCl_3$: Avoid or redistill material that comes n containers with metal-lined caps.

x. Methanol CH_3OH, reagent grade

xi. Nitric acid: HNO_3, conc.

xii. Hydrochloric acid: HCl, conc.

28.5.5 Procedure

Preparation of calibration curve: Pipette 50mL water into a 125mL separatory funnel for use as a reagent blank. Prepare standards by pipetting 1 to 10mL (20.0 to 200µg Cu) standard copper solution into a series of 125mL separatory funnels, and dilute to 50mL with water. Add 1mL of conc. H_2SO_4 and use the extraction procedure.

Construct a calibration curve by plotting absorbance versus micrograms of copper. To prepare a calibration curve for smaller amounts of copper, dilute 10mL standard copper solution through the previously described procedure, but use 5cm cells to measure absorbance.
Treatment of sample:

Transfer 100mL sample to a 250mL beaker, add 1mL conc. H_2SO_4 and 5mL conc. HNO_3. Add a few boiling chips and cautiously evaporate to dense white SO_3 fumes on a hot plate. If solution remains coloured, cool, add another 5mL conc. HNO_3, and again evaporate to dense white fumes. Repeat, if necessary, until solution becomes colourless.

Cool, add about 80mL water, and bring to a boil. Cool and filter into a 100mL volumetric flask. Make up to 100mL with water using mostly beaker and filter washings.

Pipette 50mL or other suitable portion containing 4 to 200µg Cu, from the solution obtained from preliminary treatment, into a 125mL separatory funnel. Dilute, if necessary, to 50mL with water. Add 5mL $NH_2OH.HCl$ solution and 10mL sodium citrate solution, and mix thoroughly. Adjust pH to approximately 4 by adding 1mL increments of NH_4OH until pH test paper indicates a value between 4 and 6.

Add 10mL neocuproine reagent and 10mL $CHCl_3$. Stopper and shake vigorously for 30s or more to extract the copper neocuproine complex into the $CHCl_3$ layer into a 25mL

volumetric flask, taking care not to transfer any of the aqueous layer. Repeat extraction of the water layer with an additional 10mL $CHCl_3$ and combine extracts. Dilute combined extracts to 25mL with CH_3OH, stopper, and mix thoroughly.

Transfer an appropriate portion of extract to a suitable absorption cell (1cm for 40 to 200 µg Cu, 5cm for lesser amounts) and measure absorbance at 457nm or with a 450 to 460nm filter. Use a sample blank prepared by carrying 50mL water through the complete digestion and analytical procedure.

Determine micrograms copper in final solution by reference to the appropriate calibration curve.

28.5.6 Calculation

mg Cu/L = µg Cu (in 25mL final volume) / mL portion taken for extraction

28.5.7 Bibliography

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Estimation of Metals

28.6

A. Inductively coupled plasma (ICP) method

28.6.1 Scope and application

Emission spectroscopy using inductively coupled plasma is rapid, sensitive and conventional method for the determination of metals in water and wastewater samples. Dissolved metals are determined after appropriate digestion. Care must be taken to ensure that potential interference is dealt with, especially when dissolved solids exceed 1500 mg/L.

28.6.2 Equipment

i. ICP source: The ICP source consists of a radio frequency (RF) generator capable of generating at least 1.1 KW of power, torch, tesla coil, impedance matching network, nebuliser, spray chamber, and drain. High-quality flow regulators are required for both the nebuliser argon and the plasma support gas flow. A peristaltic pump is recommended to regulate sample flow to the nebuliser. The type of nebuliser and spray chamber used may depend on the sample to be analysed as well as on the equipment manufacture. In general, pneumatic nebuliser of the concentric or cross-flow design are used. Viscous samples and samples containing particulates or high dissolved solids content may require nebulisers of the Babington type.

ii. Spectrometer: The spectrometer may be of the simultaneous or sequential type with air-part, inert gas pruged, or vacuum optics. A spectral band-pass of 0.05 nm or less is required. The Instrument should permit examination of the spectral background surrounding the emission lines used for metals determination. It is necessary to be able to measure and correct for spectral background at one or more positions on either side of the analytical lines.

28.6.3 Reagents and standards

Use reagents that are of ultra-high-purity grade or equivalent. Redistilled acids are acceptable. Dry all salts at 105°C for 1 h and store in a desiccator before weighing. Use deionised water prepared by passing water through at least two stages of deionization with mixed bed cation/anion exchange resins. Use deionised water for preparing all calibration standards, reagents and for dilution.

i. Hydrochloric acid, HCl, conc. and 1+1

ii. Nitric acid, HNO_3, conc.

iii. Nitric acid, HNO_3, 1+1: Add 500 mL conc. HNO_3, to 400 mL water and dilute to make 1L.

iv. Standard solutions:

Element	Standard solution for ICP
Aluminium	Dissolve 0.100g aluminium metal in an acid mixture of 4mL 1+1 HCl and 1mL conc. HNO_3 in a beaker. Warm gently to effect solution. Transfer to 1 L flask, add 10 mL (1+1) HCl and dilute to 1000 mL with water; 1.00 mL = 100µg Al.
Arsenic	Dissolve 1.534 g arsenic pentoxide As_2O_5 in distilled water containing 4 g NaOH. Dilute to 1000 mL; 1.00mL = 1.00 mg As (V)
Cadmium	Dissolve 0.100 g cadmium metal in 4 mL conc. HNO_3. Add 8 mL conc. HNO_3 and dilute to 1000 mL with water; 1.00 mL = 100 µg Cd.
Chromium	Dissolve 0.1923 g CrO_3 in water. When solution is complete, acidify with 10 mL conc. HNO_3 in a beaker and dilut e to 1000 mL with water; 1.00 mL = 100 µg Cr.
Copper	Dissolve 0.100 g copper metal in 2 mL conc. HNO_3, add 10.0 mL (1+1) HCl and dilute to 1000 mL with water; 1.00 mL = 100 µg Cu.
Iron	Dissolve 0.100 g iron wire in a mixture of 10 mL (1+1) of HCl and 3 mL conc. HNO_3. Add 5 mL conc. HNO_3 and dilute to 1000 mL with water; 1.00 mL = 100 µg Fe.
Lead	Dissolve 0.1598 g lead nitrate, $Pb(NO_3)_2$, in a minimum amount (1+1) of HNO_3 and dilute to 1000 mL with water; 1.00 mL = 100 µg Pb.
Manganese	Dissolve 0.1000 g manganese metal in 10 mL co nc. HCl mixed with 1 mL of conc. HNO_3 and dilute to 1000 mL = 100 µg Mn.
Nickel	Dissolve 0.1 g Ni metal in 10 mL hot conc. HNO_3, cool and dilute to 1000 mL water; 1.00 mL = 100 µg Ni.
Potassium	Dissolve 0.1907 g potassium chloride, KCl, (dried at 110 C) in water and make up to 1000 mL; 1.00 mL = 100 µg K.
Sodium	Dissolve 0.2542 g Sodium chloride, NaCl, dried at 14°C, in water and at 10 mL conc. HNO_3. And make up to 1000 mL; 1.00 Ml = 100 µg Na.
Selenium	Dissolve 2.393 g sodium selenite, Na_2SeO_4, in water containing 10 mL conc. HNO_3. Dilute to 1 L; 1.00 mL = mg Se (VI).
Zinc	Dissolve 0.10 g zinc metal in 20 mL 1+1 of HCl and dilute to 1000 mL with water; 1 mL = 100 µg Zn.

Caution: Many metal salts are extremely toxic and may be fatal, if swallowed. Wash hands thoroughly after handling.

Secondary standard: The standard solutions for metals are available with supplier, or otherwise arrange the standard prepared by recognised laboratory. Use these standards to verify the prepared standards.

i. Calibration standards

In 100 mL volumetric flasks add 2 mL of (1+1) HNO_3 and 10 mL of 1+1 HCl and dilute to 100 mL with water. Before preparing mixed standards, analyse each stock solution separately to determine possible spectral interference or the presence of impurities. When preparing mixed standards take care that the elements are compatible and stable. Store mixed standard solutions in an FEP fluorocarbon (Teflon) or unused polyethylene bottle. Verify calibration standards initially using the quality control standard; monitor weekly for stability.

ii. Calibration blank:

Dilute 2 mL of (1+1) HNO_3 and 10 mL of (1+1) HCl to 100 mL with water. Prepare a sufficient quantity to be used to flush the system between standard and samples.

iii. Method blank:

Carry a reagent blank through entire sample preparation procedure. Prepare method blank to contain the same acid types and concentrations as the sample solution.

iv. Instrument check standard:

Prepare instrument check standard by combining compatible elements as a concentrations of 2 mg/L.

v. Instrument quality control sample:

Certified aqueous reference standards are available. Use the same standards for the calibration.

vi. Argon: Use technical or welder's grade.

28.6.4 Procedure

Sample preparation: Use digested sample as described in section32.8.

i. Operating conditions: Establish instrumental detection limit, precision, optimum background correction positions, linear dynamic range and interference for each

analytical line. Verify that the instrument configuration and operating conditions satisfy the analytical requirements. An atom-to-ion emission intensity ratio (Cu (I) 324.75 nm/Mn (II) 257.61 nm) can be used to reproduce optimum conditions for multi element analysis precisely. The Cu/Mn intensity ratio may be incorporated into the calibration procedure, including specifications for sensitivity and for precision.

ii. Instrumental calibration: Warm up for 30 min. For polychromators, perform an optical alignment using the profile lamp or solution. Check alignment of plasma torch and spectrometer entrance slit. Make Cu/Mn or similar intensity ratio adjustment. Calibrate instrument using calibration standards and blank. Aspirate the standard or blank for a minimum of 15 s after reaching the plasma before beginning signal integration. Rinse with calibration blank or similar solution for at least 60 s between each standard to eliminate any carryover from the previous standards. Use average intensity of multiple integration of standards or samples to reduce random error. Before analysing samples, ensure the instrument check that concentration values obtained should not deviate from the actual values by more than ± 5%. The wavelength for the element are as suggested below. (Table 28.6.4: I)

Table 28.6.4: I: Suggested wavelength, detection limit and upper limit concentration for elements

Element	Suggested Wavelength nm	Estimate Detection Level µg/L	Alternate Wavelength nm	Calibration Concentration mg/L	Upper Limit Concentration mg/L
Aluminium	308.22	40	237.32	10.0	100
Arsenic	193.70	50	189.04	10.0	100
Cadmium	226.50	4	214.44	2.0	50
Chromium	267.72	7	206.15	5.0	50
Copper	324.75	6	219.6	1.0	50
Iron	259.94	7	238.20	10	100
Lead	220.35	40	217	10	100
Manganese	257.61	2	294.92	2	50
Nickel	231.60	15	221.65	2	50
Potassium	766.49	100	269.90	10.0	100
Sodium	589.0	30	589.59	10.0	100
Selenium	196.03	75	203.99	5.0	100
Zinc	213.86	2	206.20	5.0	100

iii. Analysis of samples: Analyse the samples using calibration blank. This permits a check of the sample preparation regents and procedures for contamination. Analyse samples, alternatively with analyses of calibration blank. Rinse for at least 60s with dilute acid between samples and blanks. After introducing each sample or blank let system equilibrate before starting signal integration. Examine each analysis of the calibration blank to verify that no carryover memory effect has occurred. If carryover is observed, repeat rinsing until proper blank values are obtained. Make appropriate dilutions of the sample to determine concentrations beyond the linear calibration.

iv. Spike test: To the known volume of digested sample, add known volume of the standard. Shake well and aspirate through ICP. Check the increase in concentration of metal for added quality of standards.

Instrument quality control: Analyse instrument check standard to confirm proper recalibration. Reanalyse one or more samples analysed just before termination of the analytical run. Results should agree with 15% error. Analyse instrument quality control sample within everyone run. Use this analysis to verify accuracy and validity of the calibration standards.

28.6.5 Calculations

Blank correction: Subtract result of an adjacent calibration blank from each sample result to make a baseline drift correction. Use the result of the method blank analysis to correct for reagent contamination.

Dilution correction: If the sample was diluted or concentrated in preparation, multiply result by a dilution factor (DF) calculated as follows:

DF =

Reporting data: report analytical data in concentration units of mg/L.

28.6.6 Bibliography

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B. Flame atomic absorption spectrophotometry

28.6.7 Scope and application

In flame atomic absorption spectrometry, a sample is aspirated into a flame and atomized. A light beam is directed through the flame, into a monochromator, and onto a detector that measures the amount of light absorbed by the atomized element in the flame. For some metal, atomic absorption exhibits superior sensitivity over flame emission. Because each

metal has got its own characteristic absorption wavelength a source lamp composed of the elements is used, this marks the method relatively free from spectral or radiation interference. The amount of energy at the concentration of the element in the sample over a limited concentration range. Most atomic absorption instruments also are equipped for operation in an emission mode, which may provide better linearity for some elements.

28.6.8 Interferences

Chemical interference:

Many metals can be determined by direct aspiration of sample into an air-acetylene flame. The most troublesome type of interference is termed "chemical" and results from the lack of absorption by atoms bound in molecular combination in the flame. This can occur when the flame is not hot enough to dissociate the molecules or when the dissociated atom is oxidised immediately to a compound that will not dissociate further at the flame temperature.

Background correction:

Molecular absorption and light scattering caused by solid particles in the flame can cause erroneously high absorption values resulting in positive errors. When such phenomena occur, use background correction to obtain accurate values. Use any one of three types of background correction:

1. Continuum-source Background correction: Continuum-source background corrector utilizes either a hydrogen filled hollow cathode lamp with metal cathode or a deuterium arc lamp. When both line sources are placed in the same optical path and are time-shared, the broadband background from the elemental signal is subtracted electronically, and the resultant signal will be background-compensated.
2. Zeeman background correction. This correction is based on the principle that a magnetic field splits the spectral in to two linearly polarized light beams parallel and perpendicular to the magnetic field. One is called the pi component and other sigma component. These tow line beams have exactly the same wavelength and differ only in the plane of polarisation. Zeeman background correction provides accurate background correction at much higher absorption levels than is possible with continuum sources background correction system.
3. Smith-Hieftje background correction: This correction is based on the principle that absorbance measured for a specific element is reduced as the hollow cathode lamp is increased while absorption of non-specific absorption substance remains identical at all current levels. When this method is applied, the absorption at a high current mode is subtracted from the absorption at a low-current mode. Under these conditions, any absorbance due to non-specific background is subtracted out and corrected for.

28.6.9 Apparatus and equipment

i. Atomic absorption spectrometer: It consists of a light source emitting the line

spectrum of an element, a device for vaporizing the sample, a means of isolating an absorption line, and a photoelectric detector with its associated electronic amplifying and measuring equipment.

ii. Burner: The most common type of burner is a premix, which introduces the spray into a condensing chamber for removal of large droplets. The burner may be fitted with a conventional head containing a single slot: a tree-slot boling head which may be preferred for direct aspiration with an air-acetylene flame, or a special head for use with nitrous oxide and acetylene.

iii. Readout: Most instruments are equipped with either a digital or null meter readout mechanism. Most modem instruments are with microprocessor or stand-alone control computers capable of integrating absorption signals over at high concentrations.

iv. Lamps: Use either a hollow-cathode lamp or electrode less discharge lamp (EDL). Use one lamp for each element being measured. Multi-element hollow-cathode lamps generally provide lower sensitivity than single element lamps. EDLs take a longer time to warm up and stabilise.

v. Pressure reducing valves: Maintain supplies of fuel and oxidant at pressures somewhat higher than the controlled operating pressure of the instrument by using suitable reducing valves. Use a separate reducing value for each gas.

vi. Vent: Place a vent about 15 to 30cm above the burner to remove fumes and vapours from the flame. This precaution protects laboratory personnel from vapours, protects the instrument from corrosive vapours, and prevents flame stability from being affected by room drafts. A damper of variable speed blower is desirable for modulating airflow and preventing flame disturbance, select blower size to provide the airflow recommended by the instrument manufacturer. In laboratory locations with heavy particulate air pollution, use clean laboratory facilities.

28.6.10 Reagents and standards

Air-Air is cleaned and dried through a suitable filter to remove oil, water, and other foreign substances. The sources may be a compressor or commercially bottled gas.
Acetylene: Standard commercial grade in which acetone is always present in acetylene cylinders. This prevents the entering and damaging the burner head by replacing a cylinder when its pressure has fallen to 689kPa (100psi) acetylene.

Caution: Acetylene gas represent an explosive hazard in the laboratory. Follow instrument manufacturer's direction in plumbing and using this gas. Do no allow gas contact with copper, brass with >65% copper, silver, or liquid mercury; do not use copper or brass tubing, regulators, or fittings.

Nitrous oxide (for Aluminium): The gas is commercially available in cylinders. Fit nitrous oxide cylinder with a special non-freezable regulator or wrap a heating coil around an ordinary regulator to prevent flashback of the burner caused by regulation in nitrous oxide flow through a frozen regulator.

Caution: Use nitrous oxide with strict adherence to manufacturer's directions. Improper sequencing of gas flow at start-up and shutdown of instrument can produce explosions from flashback.

Metal-free water: Use metal-free water for preparing all reagents and calibration standards and as dilution waste. Prepare metal-free water by deionising tap water and/or by using one of the following processes, depending on the metal concentration in the sample: single distillation, reinstallation, or sub-boiling.

Standard solution: Prepare standard solutions of known metal concentrations as described in section 32.6.3. Stock standard solution can be obtained from several commercial sources that should be used as secondary standard solution for calibration of instrument and the prepared standards.

28.6.11 Procedure

Sample preparation: Use digested sample as described in refer section 28.11.

Operating conditions: Install a hollow cathode lamp for the desired metal in the instrument and roughly set the wavelength dial according to table 28.6.11: I given below.

Table 28.6.11: I: Wavelength instrument detection level sensitivity and optimum concentration range for elements.

Element	Wavelength (nm)	**Flame Gases**	Instrument Detection Level mg/L	**Sensitivity**	Optimum Concentration range mg/L
Al	328.1	N-Ac	0.1	1	5-100
Cd	228.8	A-Ac	0.002	0.025	0.05-2
Cr	357.6	A-Ac	0.2	0.1	0.2-10
Cu	324.7	A-Ac	0.1	0.1	0.2-10
Fe	248.3	A-Ac	0.02	0.12	0.3-10
Mn	279.5	A-Ac	0.01	0.05	0.1-10
Ni	232.0	A-Ac	0.02	0.15	0.3-10
Pb	283.3	A-Ac	0.05	0.5	1-20
Zn	213.9	A-Ac	0.005	0.02	0.05-2

A-Ac = air-acetylene; N-Ac = nitrous oxide-acetylene.

Set slit width according to manufacturer-suggested setting for the element being measured. Turn on instrument; apply to the hollow-cathode lamp the current suggested by the manufacturer, and let instrument warm-up until energy source stablilises, generally about 10 to 20 min. Readjust current as necessary after warm-up. Optimise wavelength by adjusting wavelength dial until optimum energy gain is obtained. Align lamp in accordance with manufacturer's instruction.

Install suitable burner head and adjust burner head position. Turn on air and adjust flow rate to that specified by manufacturer to give maximum sensitivity for the metal being measured. Turn on acetylene, adjust flow rate to value specified, and ignite flame. Let flame stabilise for a few minutes. Aspirate a blank consisting of deionised water containing the same concentration of acid in standard and samples. Adjust the instrument to zero, aspirate a standard solution and adjust aspiration rate of the nebuliser to obtain maximum sensitivity. Adjust burner both vertically and horizontally to obtain maximum response. Aspirate blank again and check zero reading of the instrument, Aspirate a standard near the middle of the linear range. Record absorbance of this standard when freshly prepared and with a new hollow cathode lamp. Refer to the data on subsequent determination of the same element to check consistency of instrument setup and aging of hollow cathode lamp and standard.

The instrument now is ready to operate. When analysis is finished extinguish flame by turning off first acetylene and then air.

Standardisation: Select at least three concentrations of each standard metal solution to bracket the expected metals concentration of a sample. Aspirate blank and adjust zero of the instrument. Then aspirate each standard in turn into flame record absorbance.

Prepare a calibration curve by plotting on linear graph paper absorbance of standard versus their concentrations. For instruments equipped with direct concentration readout, this step is unnecessary. With some instrument it may be necessary to convert percent absorption by using a table generally provided by the manufacturer.

28.6.12 Calculations

Calculate concentration of each ion, in µg/L for trace elements, and in mg/L for more common metals, by referring to the appropriate calibration curve. Alternatively read concentration directly from the instrument readout if the instrument is so equipped. If the sample has high values multiply it by the appropriate dilution factor.

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28.7 Iron (Fe)

28.7.1 Scope and equipment

Iron occurs in the minerals as hematite, taconite and pyrite. It is widely used in steel and other alloys. Elevated iron levels in water can cause stains in plumbing, laundry and cooking utensils and can impart objectionable taste and colour to foods. The United Nations FAO recommended level for irrigation water is 5mg/L. The US EPA secondary drinking water standard MCL is 0.3mg/L. The BIS standard desirable limit is 0.3mg/L.

Methods for analysis:

A. Inductively coupled plasma (ICP) method/AAS

B. Phenanthroline method

A. Inductively coupled plasma method

Please refer section 28.6 A.

B. Phenanthroline method

Principle: Iron is brought into solution, reduced to the ferrous state by boiling with acid and hydroxylamine, and treated with 1, 10-phenanthroline at pH 3.2 to 3.3. Three molecules of Phenanthroline chelate each atom of ferrous iron to form an orange-red complex. The coloured solution obeys Beer's law; its intensity is independent of pH from 3 to 9. A pH between 2.9 and 3.5 insures rapid colour development in the presence of an excess of Phenanthroline. Colour standards are stable for at least 6 months.

Interference: Among the interfering substances are strong oxidising agents, cyanide, nitrite, and phosphates (polyphosphates more so than orthophosphate), chromium, zinc in concentrations exceeding 10 times that of iron, cobalt and copper in excess of 5mg/L, and nickel in excess of 2mg/L. Bismuth, cadmium, mercury, molybdate, and silver precipitate Phenanthroline. The initial boiling with acid converts polyphosphates to orthophosphate and removes cyanide and nitrite that otherwise would interfere. Adding excess hydroxylamine eliminates errors caused by excessive concentrations of strong oxidising reagents. In the presence of interfering metal ions, use a larger excess of Phenanthroline to replace that complexed by the interfering metals. Where excessive concentrations of interfering metal

ions are present, the extraction method may be used. If noticeable amounts of colour or organic matter are present, it may be necessary to evaporate the sample, gently ash residue, and redissolve in acid. Ashing may be carried out in silica, porcelain crucibles that have been boiled for several hours in 1+1 HCl. The presence of excessive amounts of organic matter may necessitate digestion before use of the extraction procedure.

Minimum detectable concentration: Dissolved or total concentrations of iron as low as 10µg/L can be determined with a spectrophotometer using cells with a 5 cm or longer light path. Carry a blank through the entire procedure to allow for correction.

28.7.2 Apparatus and equipment

i. Spectrophotometer: use a 510nm, providing a light path of 1cm or longer.

ii. Acid-washed glassware: wash al glassware with conc. hydrochloric acid (HCl) and rinse with distilled water before use to remove deposits of iron oxide.

iii. Separatory funnels: 125mL, squibb from, with ground glass or TFE stopcocks and stoppers.

28.7.3 Reagents and standards

Use reagents and distilled water free from iron contamination

i. Hydrochloric acid, HCl, conc. containing less than 0.5 ppm iron.

ii. Hydroxylamine solution: Dissolve 10g $NH_2OH.HCl$ in 100mL water.

iii. Ammonium acetate buffer solution: Dissolve 250g $NH_4C_2H_3O_2$ in 150mL water. Add 70mL conc. (glacial) acetic acid. Because even a good grade of $NH_4C_2H_3O_2$ contains a significant amount of iron, prepare new reference standards with each buffer preparation.

iv. Sodium acetate solution: Dissolve 200g $NaC_2H_3O_2.3H_2O$ in 800 mL water.

v. Phenanthroline solution: Dissolve 100mg 1, 10-phenanthroline monohydrate, $C_{12}H_8N_2.H_2O$, in 100mL water by stirring and heating to 80°C. Do not boil. Discard the solution if it darkens. Heating is unnecessary if 2 drops of conc. HCl are added to the water (note: One milliliter of this reagent is sufficient for no more than 100µg Fe).

vi. Stock iron solution: Use metal (1) or salt (2) for preparing the stock solution.

   i. Use electrolytic iron wire, or "iron wire for standardizing" to prepare the solution. If necessary, clean wire with fine sandpaper to remove any oxide coating and to produce a bright surface. Weigh 200mg wire and place in a 1000mL volumetric flask. Dissolve in 20mL 6N sulphuric acid (H_2SO_4) and dilute to mark with water; 1mL = 200µg Fe.

ii. If ferrous ammonium sulphate is preferred, slowly add 20mL conc.H_2SO_4 to 50mL water and dissolve 1.404g Fe $(NH_4)_2$ $(SO_4)_2$.$6H_2O$.Add 0.02M potassium permanganate (KMnO4) drop-wise until a faint pink colour persists. Dilute to 1000mL with water and mix; 1mL = 200µg Fe.

Standard iron solutions: Prepare daily for use.

- Pipette 50mL stock solution into a 1000 mL volumetric flask and dilute to mark with water; 1mL = 10µg Fe.
- Pipette 5mL stock solution into a 1000 mL volumetric flask and dilute to mark with water; 1 mL = 1µg Fe.

vii. Disopropyl or isopropyl ether.

Cautions: Ethers may form explosive peroxides; test before using.

28.7.3 Procedure

Total iron: Mix sample thoroughly and measure 50mL into a 125mL Erlenmeyer flask. If this sample volume contains more than 200 µg iron, use a smaller accurately measured portion and dilute to 50mL. Add 2mL conc. HCl and 1mL $NH_2OH.HCl$ solution. Add a few glass beads and heat to boiling. To insure dissolution of all the iron, continue boiling until volume is reduced to 15 to 20mL. (If the sample is ashed, take up residue in 2mL conc. HCl and 5mL water). Cool to room temperature and transfer to a 50 or 100 mL volumetric flask or nessler tube. Add 10mL $NH_4C2H_3O_2$ buffer solution and 4mL Phenanthroline solution and dilute to mark with water. Mix thoroughly and allow at least 10 to 15 min to maximum colour development.

Dissolved iron: Immediately after collection filter sample through a 0.45µg membrane filter into a vacuum flask containing 11mL conc. HCl/ferrous iron. Calculate suspended iron by subtracting dissolved from total iron.

Ferrous iron: Determine ferrous iron at sampling site because of the possibility of change in the ferrous-ferric ration with time in acid solutions. To determine ferrous iron only, acidify a separate sample with 2mL conc. HCl/100mL sample at the time of collection. Fill bottle directly from sampling source and stopper. Immediately withdraw a 50mL portion of acidified sample and add 20mL Phenanthroline solution and 10mL $NH_4C_2H_3O_2$ solution with vigorous stirring. Dilute to 100mL and measure colour intensity within 5 to 10 min. Do not expose to sunlight. (Colour development is rapid in the presence of exc Phenanthroline. The Phenanthroline volume given is suitable for less than 50µg total iron, if larger amounts are present, use a correspondingly larger volume of Phenanthroline or a more concentrated reagent. Excess Phenanthroline is required because of kinetics of the complexing process). Calculate ferric iron by subtracting ferrous from total iron.

Colour measurement: Prepare a series of standards by accurately pipetting calculated

volumes of standard iron solutions (use weaker solution to measure 1 to 10µg portions) into 125mL Erlenmeyer flasks, diluting to 50mL by adding measured volumes of water.

For photometric measurement, use light path at 510nm. Read standards against distilled water set at zero absorbance and plot a calibration curve, including a blank.

If samples are coloured or turbid, carry a second set of samples through all steps of the procedure without adding Phenanthroline.

Samples containing organic interferences: Digest samples containing substantial amounts of organic substances.

a. From the digested sample, pipette 10.0 mL or other suitable portion containing 20 to 500µg Fe into a 125mL separatory funnel. If the volume taken is less than 10mL, add distilled water to make up to 10mL. To the separatory funnel add 15 mL conc. HCl for a 10mL aqueous volume; or, if the portion taken was greater than 10mL. Add 1.5mL conc. HCl/mL of sample. Mix, cool.

b. To prepare a sample solely for determining iron, measure a suitable volume containing 20 to 500µg Fe. However, use only 5mL of H_2SO_4 or $HClO_4$ and omit H_2O_2. When digestion is complete, cool, dilute with 10mL of water, heat almost to boiling to dissolve slowly soluble salts and, if the sample is still cloudy, filter through a glass fibre, sintered-glass or porcelain filter, washing with 2 to 3 mL water. Quantitatively transfer filtrates or clear solution to a 25mL volumetric flask and make up to 25mL with water. Empty flask into a 125mL separatory funnel, rinse with 5mL conc. HCl that is added to the funnel and add 25mL conc. HCl measured with the same graduated or flask. Mix and cool to room temperature.

c. Extracts iron from the HCl solution in the separatory funnel by shaking for 30s with 25mL isopropyl ether (caution). Draw off lower acid layer into a second separatory funnel. Extract acid solution again with 25mL isopropyl ether, drain acid layer into a suitable clean vessel and combine the two portions of isopropyl ether. Pour acid layer back into second separatory funnel and re-extract with 25 mL isopropyl ether. Withdraw and discard acid layer and add ether layer to original funnel. Persistance of a yellow colour in the HCl solution after three extractions does not signify incomplete separation of iron because copper, which is not extracted, gives a similar yellow colour. Shake combined ether extracts with 25mL water to return iron to aqueous phase and transfer lower aqueous extract. Discard ether layer.

d. Add 1mL $NH_2OH.HCl$ solution, 10mL Phenanthroline solution and 10mL $NaC_2H_3O_2$ solution. Dilute to 100mL with water, mix thoroughly and let stand for 10 min. Measure absorbance at 510 nm using a 5cm absorption cell for amounts of iron less than 100 µg or 1cm cell for quantities form 100 to 500µg. As reference, use either distilled water or a sample blank prepared by carrying the specified quantities of acids through the entire analytical procedure. If distilled water is used as reference, correct sample absorbance by subtracting absorbance of a sample blank. Determine micrograms of iron in the sample from the absorbance (corrected, if necessary) by

reference to the calibration curve prepared by using a suitable range of iron standards containing the same amounts of Phenanthroline, hydroxylamine and sodium acetate as the sample.

28.7.4 Calculation

mg Fe/L = µg Fe (in 100mL final volume) / mL sample

OR for digested samples

Mg Fe/L = {µg Fe (in 100mL final volume) / mL sample} x {100 / mL portion}

Report details of sample collection, storage and pre-treatment, if they are pertinent to interpretation of results.

28.7.5 Bibliography

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Lead (Pb)

28.8

28.8.1 Scope and application

Lead in a water supply may come form industrial, smelter discharges and mine or from the dissolution of plumbing and plumbing fixture. Tap water that are inherently not corrosive or not suitably treated may contain lead resulting from an attack on lead service pipes, lead interior plumbing, brass fixtures and fittings on solder pipe joints chiefly from galena (PbS). It is used in batteries, ammunition, solder, piping, pigments, insecticides and alloys. Lead also was used in gasoline for many years as an anti-knock agent in the form of tetraethyl lead. The Food and drug administration regulates lead content in food and in house paints. Under the lead-copper rule, the US EPA drinking water standard for lead is 15µg/L. The BIS desirable limit is 0.05mg/L.

Methods for analysis:

A. Inductively coupled plasma method/AAS

B. Dithizone method

A. Inductively coupled plasma method

Please refer section 28.6 A.

B. Dithizone method

28.8.2 Summary of the method

Principle: An acidified sample containing microgram quantities of lead is mixed with ammonical citrate-cyanide reducing solution and extracted with dithizone in chloroform ($CHCl_3$) to form a cherry-red lead dithizonate. The colour of the mixed colour solution is measured photometrically. Sample volume taken for analysis may be 2L when digestion is used.

Interference: In a weekly ammonical cyanide solution (pH 8.5 to 9.5) dithizone forms coloured complexes with bismuth, stannous tin and monovalent thallium. In strongly ammonical citrate-cyanide solution (pH 10 to 11.5) the dithizonates of these ions are unstable and are extracted only partially. This method uses a high pH, mixed colour and

single dithizone extraction. Interference from stannous tin and mono-valent thallium is reduced further when these ions are oxidised during preliminary digestion. A modification of the method allows detection and eliminate of bismuth interference. Excessive quantities of bismuth, thallium and tin may be removed.

Dithizone in $CHCl_3$ absorbs at 510nm; control its interference by using nearly equal concentrations of excess dithizone in samples, standards and bland. The method is without interference for the determination of 0 to 30µg Pb in the presence of Tl^{+}, 100 µg Sn^{2+}, 200µg In^{3+} and 1000µg each of Ba^{2+}, Cd^{2+}. Cp^{2+}, Cu^{2+}, Mg^{2+}, Mn^{2+}, Hg^{2+}, Sr^{2+}, Zn^{2+}, Al^{3+}, Sb^{3+}, As^{3+}, Cr^{3+}, Fe^{3+}, V^{3+}, PO_4^{3-} and SO_4^{2-}. Gram quantities of alkali metals do not interfere. A modification is provided to avoid interference from excessive quantities of bismuth or tin.

Preliminary sample treatment: At the time of collection acidify with conc. HNO_3 to pH <2 but avoid excess HNO_3. Add 5mL of 0.01N iodine solution to avoid losses of volatile organo-lead compounds during handling and digestion of samples. Prepare a blank of lead-free distilled water and carry through the procedure.

Digestion of samples: Digest the samples if necessary.

Minimum detectable concentration: 1.0µg Pb/10mL dithizone solution.

28.8.3 Apparatus and equipment

i. Spectrophotometer: for use at 510nm, providing a light path of 1cm or longer.

ii. pH meter

iii. Separatory funnels: 250mL Squibb type. Clean all glassware, including sample bottles, with 1+1 HNO_3. Rinse thoroughly with distilled or deionised water.

iv. Automatic dispensing burettes: Use for all reagents to minimise indeterminate contamination errors.

28.8.4 Reagents and standards

Use reagents and distilled water free from aluminium contamination

i. Stock lead solution: Dissolve 0.1559g lead nitrate, Pb(NO3)2, (minimum purity 99.5%) in approximately 200mL water. Add 10 mL conc. HNO_3 and dilute to 1000mL with water. Alternatively, dissolve 0.1000g pure Pb metal in 20mL 1+1 HNO_3 and dilute to 1000mL with water; 1mL = 100µg Pb.

ii. Working lead solution: Dilute 2.0mL stock solution to 100mL with water; 1mL = 2.00µg Pb.

iii. Nitric acid: HNO_3, (1+4) Dilute 200mL conc. HNO\ to 1L with water.

iv. Ammonium hydroxide; NH_4OH, (1+9): Dilute 10mL conc. NH_4OH to 100mL with water.

v. Citrate-cyanide reducing solution: Dissolve 400g dibasic ammonium citrate, $(NH_4)_2\ HC_6H_5O_7$, 20g anhydrous sodium sulphite, Na_2SO_3, 10g hydroxylamine hydrochloride, $NH_2OH.HCl$ and 40g potassium cyanide, KCN (caution: poison) in water and dilute to 1L. Mix this solution with 2L conc. NH_4OH. Do not pipette by mouth.

vi. Stock dithizone solution: As per the procedure described in cadmium method.

vii. Dithizone working solution: Dilute 100mL stock dithizone solution to 250mL with $CHCl_3$; 1mL = 40µg dithizone.

viii. Special dithizone solution: Dissolve 250mg dithizone in 250mL $CHCl_3$. This solution may be prepared without purification because all extracts using it are discarded.

ix. Sodium sulphite solution: Dissolve 5g anhydrous Na_2SO_3 in 100mL water.

x. Iodine solution: Dissolve 40g KI in 25 mL water, add 12.7g resublimed iodine and dilute to 1000 mL.

28.8.5 Procedure

With sample digestion: To a digested sample containing not more than 1 mL conc. acid add 20mL of (1+4) HNO_3 and filter through lead-free filter paper (Whatman No. 42 or equivalent) and filter funnel directly into a 250mL Separatory funnel. Rinse digestion beaker with 50 mL water and add to filter. Add 50mL ammoniacal citrate-cyanide solution, mix and cool to room temperature. Add 10mL dithizone working solution, shake stoppered funnel vigorously for 30s and let layers separate. Insert lead-free cotton in stem of Separatory funnel and draw off lower layer. Discard 1 to 2mL $CHCl_3$ layer, then fill absorption cell. Measure absorbance of extract at 510nm, using dithizone-working solution, to zero spectrophotometer.

Without sample digestion: To 100mL acidified sample (pH=2) in a 250 mL separatory funnel add 20mL of (1+4) HNO_3 and 50mL citrate-cyanide reducing solution; mix. Add 10mL dithizone working solution and proceed.

Calibration curve: Plot concentration of at least five standards and a blank against absorbance. Determine concentration of lead in extract from curve. All concentrations are µg Pb/10mL final extract.

Removal of excess interferences: The dithizonates of bismuth, tin and thallium differ from lead dithizonate in maximum absorbance. Detect their presence by measuring sample absorbance at 510n, and at 465nm. Calculate corrected absorbance of sample at each wavelength by subtracting absorbance of blank at same wavelength. Calculate ration of

corrected absorbance at 510nm to corrected absorbance at 465nm. The ratio of corrected absorbance for lead dithizonate is 2.08 and for bismuth dithizonate is 1.07. If the ratio for the sample indicates interference, i.e., is markedly less than 2.08, proceed as follows with a new 100mL sample: If the sample has not been digested, add 5mL Na_2SO_3 solution to reduce iodine preservative. Adjust sample to pH 2.5 using a pH meter and 1+4 HNO_3 or 1+9 NH_4OH as required. Transfer sample to 250mL separatory funnel, extract with a minimum of three 10mL portions special dithizone solution, or until the $CHCl_3$ layer is distinctly green. Extract with 20mL portions $CHCl_3$ to remove dithizone (absence of green). Add 20mL of (1+4) HNO_3, 50mL citrate-cyanide reducing solution and 10mL dithizone working solution. Extract and measure absorbance.

28.8.6 Calculation

mg Pb/L = µg Pb (in 10mL, from calibration curve) / mL sample

28.8.7 Bibliography

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Manganese (Mn)

28.9

28.9.1 Scope and application

Manganese is associated with iron minerals and occurs in nodules in ocean, fresh water and soils. The common ores are pyrolusite and psilomelane. Manganese is used in steel alloys, batteries and food additives. The FAO-recommended maximum level for manganese in standard is 50µg/L. BIS desirable limit is 0.1 mg/L.

Methods for analysis:

A. Inductively coupled plasma method

B. Persulphate Method

Sampling and storage

Manganese may exist in a soluble form in neutral water when first collected, but it oxidises to a higher oxidation state and precipitates or becomes absorbed on the container walls. Determine very soon after sample collection. When delay is unavoidable, total manganese can be determined if the sample is acidified at the first time of collection with HNO3 to pH <2.

A. Inductively coupled plasma method

Please refer section28.6 A.

B. Persulphate method

28.9.2 Summary of the method

Principle: Persulphate oxidation of soluble manganous compounds to form permanganate is carried out in the presence of silver nitrate. The resulting colour is stable for at least 24h if excess Persulphate is present and organic matter is absent.

Interferences: As much as 0.1g chloride (Cl) in a 50-mL sample can be prevented from interfering by adding 1g mercuric sulphate ($HgSO_4$) to form slightly dissociated complexes. Bromide and iodine still will interfere and only trace amounts may be present. The

Persulphate procedure can be used for potable water with trace to small amounts of organic matter, if the period of heating is increased after more Persulphate has been added.

For wastewater containing organic matter, use preliminary digestion with nitric and sulphuric acids (HNO3 and H2SO4). If large amounts of Cl^- interfering traces of Cl^- in the special reagent.
Coloured solutions from other inorganic ions are compensated for in the final colorimetric step. Samples that have been exposed to air may give low results due to precipitation of manganese dioxide (MnO_2). Add 1 drop of 30% hydrogen peroxide (H_2O_2) to the sample, after adding the special reagent, to redissolve precipitated manganese.

Minimum detectable concentration:

The molar absoptivity of permanganate ion is about 2300 Lg^{-1} cm^{-1}. This corresponds to a minimum detectable concentration (98% transmittance) of 210µg Mn/L when a 1cm cell is used or 42µg Mn/L when a 5cm cell is used.

28.9.3 Apparatus and equipment

i. Colorimetric equipment: One of the following is required: Spectrophotometer: for use at 525nm, providing a light path of 21cm or longer.

   Filter photometer: providing a light path of 1cm or longer and equipped with a green filter having maximum transmittance near 525nm.

ii. Nessler tubes: matched, 100mL, tall form.

28.9.4 Reagents and standards

Use reagents and distilled water free from manganese contamination.

i. Special reagent: Dissolve 75g $HgSO_4$ in 400mL conc. HNO_3 and 200mL distilled water. Add 200mL 85% phosphoric acid (H_3PO_4) and 35mg silver nitrate ($AgNO_3$). Dilute the cooled solution to 1L.

ii. Ammonium Persulphate: $(NH_4)_2S_2O_8$ solid.

iii. Standard manganese solution: Prepare a 0.1N potassium permanganate ($KMnO_4$) solution by dissolving 3.2g $KMnO_4$ in distilled water and making up to 1L. Age for several weeks in sunlight or heat for several hours near the boiling point, then filter through a fine fritted-glass filter crucible and standardise against sodium oxalate as follows:

   Weigh several 100 to 200mg samples of $Na_2C_2O_4$ to 0.1mg and transfer to 400mL beakers. To each beaker, add 100mL distilled water and stir to dissolve. Add 10mL of

(1+1) H_2SO_4 and heat rapidly to 90 to 95°C. Titrate rapidly with the $KMnO_4$ solution to be standardised, while stirring, to a slight pink endpoint colour that persists for at least 1 min. Do not let temperature fall below 85°C. If necessary, warm beaker contents during titration; 100mg $Na_2C_2O_4$ will consume about 15mL, permanganate solution. Run a blank on distilled water and H_2SO_4.

Normality of $KMnO_4$ = g $Na_2C_2O_4$ / (A B) x 0.067 01

Where:

A = mL titrant for sample and
B = mL titrant for blank

Average results of several titrations; calculate volume of this solution necessary to prepare 1L of solution so that 1.00mL = 50µg Mn as follows:

mL $KMnO_4$ = 4.55 / Normality, $KMnO_4$

To this volume, add 2 to 3 mL conc. H_2SO_4 and $NaHSO_3$ solution drop wise, with stirring, until the permanganate colour disappears. Boil to remove excess SO_2, cool and dilute to 100mL with distilled water. Dilute this solution further to measure small amounts of manganese.

iv. Standard manganese solution (alternate): Dissolve 1g manganese metal (99.8% min) in 10mL redistilled HNO_3. Dilute to 1000mL with 1% (v/v) HCl; 1mL = 1mg Mn. Dilute 10mL to 200mL with distilled water; 1mL = 0.05mg Mn. Prepare dilute solution daily.

v. Hydrogen peroxide: H_2O_2, 30%

vi. Nitric acid: HNO_3, conc.

vii. Sulphuric acid: H_2SO_4, conc.

viii. Sodium nitrite solution: Dissolve 5g $NaNO_2$ in 95mL distilled water.

ix. Sodium oxalate: $Na_2C_2O_4$, primary standard

x. Sodium bisulphate: Dissolve 10g $NaHSO_3$ in 100mL distilled water.

28.9.5 Procedure

Treatment of sample: If a digested sample has been prepared according to directions for reducing organic matter and/or excessive chlorides, pipette a portion containing 0.05 to 2mg Mn into a 250mL conical flask. Add distilled water, if necessary, to 90mL and proceed.

To a suitable sample portion add 5mL special reagent and 1 drop H_2O_2. Concentrate to 90mL by boiling or dilute to 90mL. Add 1g $(NH_4)_2S_2O_8$, bring to a boil and boil for 1min.Do not heat on a water bath. Remove from heat source, let stand 1 min and then cool under the tap (boiling too long results in decomposition of excess Persulphate and subsequent loss of permanganate colour; cooling too slowly has the same effect). Dilute to 100mL with distilled water free from reducing substances and mix. Prepare standards containing 0, 5, to 1500µg Mn by treating various amounts of standard Mn solution in the same way.
Photometric determination:

Use a series of standards from 0 to 1500µg Mn/100 ml. final volume. Make photometric measurements against a distilled water blank. The following table shows light path length appropriate for various amounts of manganese in 100ml. final volume:

Mn Range	Light Path
µg	cm
5-200	15
20-400	5
50-1000	2
100+1500	1

Prepare a calibration curve of manganese concentration vs. absorbance from the standards and elements and determine Mn in the samples from the curve.

Correction for turbidity or interfering colour:

Avoid filtration because of possible retention of some permanganate on the filter paper. If visual comparison is used, the effect of turbidity only can be estimated and no correction can be made for interfering coloured ions. When photometric measurements are made, use the following 'bleaching' method, which also corrects for interfering colour. As soon as the photometer reading has been made, add 0.05mL H_2O_2 solution permanganate colour has faded completely and no bubbles remain, absorbance to obtain absorbance due to Mn.

28.9.6 Calculation

i. When the entire original sample is taken for analysis:

   Mn, mg/L = {µg Mn (in 100mL final volume) / mL sample} x {100 / mL portion}

ii. When a portion of the digested sample (100mL final volume) is taken for analysis:

   Mn, mg/L = µg Mn / 100mL / mL sample

28.9.7 Bibliography

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28.10 Nickel (Ni)

28.10.1 Scope and application

The average abundance of Ni in the earth's crust is 1.2 ppm; in soils it is 2.5 ppm; in streams it is 1µg/L, and in groundwater it is <0.1 mg/L. Nickel is obtained chiefly from pyrrhotite and garnierite. Nickel is used in alloys, magnets, protective coating, catalysts, and batteries. The common aqueous species is ni2+. In reducing conditions insoluble sulphide can form, while in aerobic conditions insoluble sulphide can form, while in aerobic conditions ligands can from. It is suspected to be an essential trace element for some plants and animals. The standards of United Nations Food for irrigation water is 200µg/L. The US EPA primary drinking water standard MCL is 0.1 mg/L.

Methods for analysis:

A. Atomic absorption Spectrometric method.

B. Inductively coupled plasma method

A. Atomic absorption spectrometric method

Please refer section 28.6 B.

B. Inductively coupled plasma method

Please refer section 28.6 A.

Preliminary Digestion for Metals

28.11

To reduce interference by organic matter and to convert metal associated with particulate to a form (usually the free metal) that can be determined by inductively coupled plasma spectroscopy, use one of the digestion techniques. Use the least rigorous digestion method required providing complete and consistent recovery compatible with the analytical method and the metal being analysed.

- Open digestion
- Closed system digestion (Microwave-assisted digestion).

Nitric acid will digest most samples adequately. Nitrate is an acceptable matrix for both flame and electro-thermal atomic absorption. Some samples may require addition of perchloric, hydrochloric, or sulphuric acid for complete digestion. Confirm metal recovery for each digestion and analytical procedures used. As a general rule, HNO_3 alone is adequate for clean samples or easily oxidised materials; HNO_3-H_2SO_4 or HNO_3-HCl digestion is adequate for readily oxidisable organic matter; HNO_3-$HClO_4$ or HNO_3-$HClO_4$-HF digestion is necessary for difficult-to-oxidise organic matter or minerals. Dry ash formation is helpful if large amounts of organic matter are present.

28.11.1 Open digestion

A. Nitric Acid Digestion

Apparatus:

Hot plate, Conical (Erlenmeyer) flasks, 125mL or Griffin beakers 150mL, acid-washed and rinsed with double distilled water; volumetric flasks, 100mL.

Reagents:

Nitric acid, concentrated analytical grade or trace metal grade

Procedure:

Transfer a measured volume (50mL) of well-mixed, acid-preserved sample to a flask or beaker. Add 5mL conc. HNO_3 and a few boiling chips or glass beads. Bring to a slow boil and evaporate on a hot plate to the lowest volume possible (about 10 to 20mL).Continue heating

and adding conc. HNO_3 as necessary until digestion is complete as shown by a light-coloured, clear solution. Do not let sample dry during digestion.

Wash down flask or beaker walls with water and then filter if necessary. Transfer filtrate to a 10mL volumetric flask with two 5mL portions of water, adding these rinsing to the volumetric flask. Cool, dilute to mark and mix thoroughly. Take portions of this solution for required metal determinations.

B. Nitric acid-Hydrochloric acid digestion

Apparatus:

Hot plate, Conical (Erlenmeyer) flasks 125mL or Griffin beakers 150mL, acid-washed and rinsed with double distilled water, volumetric flasks 100mL, watch glasses; steam bath.

Reagents:

a. Nitric acid, HNO_3, concentrated

b. Hydrochloric acid, 1+1

Procedure:

Total metals by digestion with HNO_3/HCl: Transfer a measured volume of well mixed acid-preserved sample appropriate for the expected metal concentrations to a flask or beaker. Add 3mL conc. HNO_3.Place flask or beaker on a hot plate and cautiously evaporate to less than 5mL, making sure that the sample should not boil and that no area of the bottom of the container is allowed to go dry.

Cool and add 5mL conc. HNO_3.

Cover container with a watch glass and return to hot plate. Increase temperature of hot plate so that a gentle reflux action occurs. Continue heating, adding additional acid as necessary, until digestion is complete. Evaporate to less than 5mL and cool. Add 10mL (1+1) HCl and 15mL water per 100mL and makeup the final volume. Heat the sample for an additional 15 min to dissolve the precipitate or residue. Cool, wash down beaker walls and watch glass with water; and transfer filtrate to a insoluble materials that could clog the nebuliser; and transfer filtrate to a 100mL volumetric flask with rinsing. Alternatively centrifuge or let settle overnight. Adjust to volume and mix thoroughly.

Recoverable HNO_3/HCl: For this, transfer a measured volume of well-mixed, acid-preserved sample to a flask of beaker. Add 2mL (1+1 HNO_3) and 10mL (1+1 HCl) and heat on a steam bath of hot plate until volume has been reduced to near 25mL, making certain sample does not boil. Cool and filter to remove insoluble material or alternatively centrifuge or let settle overnight. Transfer sample to volumetric flask, adjust volume to 100mL, and mix.

C. Nitric Acid-Sulphuric Acid Digestion

Apparatus:

The same apparatus as indicated above.

Reagents:

a. Nitric acid, HNO_3, conc.

b. Sulfuric acid, H_2SO_4 conc.

Procedure:

Transfer a measured volume of well-mixed, acid-preserved sample appropriate for the expected metal concentrations to a flask or beaker. Add 5mL conc. HNO_3 and a few boiling chips, glass beads. Bring to slow boil on hot plate and evaporate to 15 to 20mL. Add 5mL conc. HNO_3 and 10mL conc. H_2SO_4. Evaporate on a hot plate until dense white fumes of SO_3 just appear. If solution does not clear, add 10mL conc. HNO_3 and repeat evaporation to fumes SO_3. Heat to remove all HNO_3 before continuing treatment. All HNO_3 will be removed when the solution is clear and no brownish fumes are evident. Do no let sample dry during digestion. Cool and dilute to about 50mL with water. Heat to almost boiling slowly to dissolve soluble salts, filter if necessary, then complete procedure as described above.

D. Nitric Acid-Perchloric Acid Digestion

Apparatus:

Same apparatus as indicated above

Safety shields, safety goggles, watch glasses.

Reagents:

a. Nitric acid, HNO_3, conc.

b. Perchloric acid, $HClO_4$,

c. Ammonium acetate solution: Dissolve 500g $NH_4C_2H_3O_2$ in 600mL water.

Cautions: Heated mixtures of $HClO_4$ and organic matter may explode violently. Avoid this hazard by taking the following precautions:

a. Do not add $HClO_4$ to a hot solution containing organic matter

b. Always pre-treat samples containing organic matter with HNO_3 before adding $HClO_4$

c. Avoid repeated fuming with $HClO_4$ in ordinary hoods)For routine operations, use a water pump attached to glass fume eradicator stainless steel fume hoods with adequate water wash down facilities are available commercially and are acceptable for use with $HClO_4$) and

d. Never let samples being digested with $HClO_4$ evaporate to dryness.

Procedure:

Transfer a measured volume of well-mixed, acid-preserved sample appropriate for the expected metal concentrations to a flask or beaker. Add 5mL conc. HNO_3 and a few boiling chips, glass beads, or Hengar granules, and evaporate on a hot plate to 15 to 20mL. Add 10mL each of conc. HNO_3 and $HClO_4$, cooling flask or beaker between additions. Evaporate gently on a hot plate until dense white fumes of $HClO_4$ just appear. If solution is not clear, cover container with a watch glass and keep solution just boiling until it clear. If necessary, add 10mL of conc. HNO_3 to complete digestion Cool, dilute to about 50mL with water, and boil to expel any chlorine or oxides of nitrogen. Filter.

E. Nitric Acid-Perchloric Acid-Hydrofluoric Acid Digestion

Apparatus:

Hot plate, TFE beakers, 250mL, acid-washed and rinsed with water, volumetric flasks 100mL, Polypropylene or other suitable plastic.

Reagents:

a. Nitric acid, HNO_3, conc. and 1+1

b. Perchloric acid, $HClO_4$

c. Hydrofluoric acid, HF, 48 to 51%.

Caution: Handle HF with extreme care and provide adequate ventilation, especially for the heated solution. Avoid all contact with exposed skin. Provide medical attention for HF burns.

Procedure:

Transfer a measured volume of well-mixed, acid preserved sample appropriate for the expected metal concentration into a 250mL TFE beaker. Evaporate on a hot plate to 1 to 20mL. Add 12mL conc.HNO_3 and evaporate to near dryness. Repeat HNO_3 addition and evaporation. Let solution cool; add 20mL $HClO_4$ and 1mL HF, and boil until solution is clear and white fumes of $HClO_4$ have appeared. Cool, add about 50mL water, filter, and make up

the volume to known concentration using volumetric flask.

F. Dry ashing

Apparatus:

Evaporating dishes: Dishes of 100mL capacity made up of one of the following materials: Porcelain (90mm dia.), Platinum-Generally satisfactory for all purposes, High-silica glass.

Muffle furnace for operation at 550 ±50°C; Steam bath; Desiccator provided with a desicant containing a colour indicator of moisture concentration; drying oven for operation at 103 to 105°C; Analytical balance, capable of weighing to 0.1mg.

Procedure:

Mix sample and transfer to a known volume into a platinum or high-silica glass-evaporating dish. Evaporate to dryness on a steam bath. Transfer dish to a muffle furnace and heat sample to a white ash. If volatile elements are to be determined, keep temperature at 400°C to 450°C. If sodium only is to be determined, ash the sample at a temperature of 600°C. Dissolve ash in a minimum quantity of conc. HNO_3 and warm water. Filter diluted sample and adjust to a known volume, preferably so that the final HNO_3 concentration is about 1%. Take portions of this solution for metal determination.

28.11.2 Microwave digestion system

Scope and application:

The microwave region of the electromagnetic spectrum lies between infrared radiation and radio frequencies and corresponds to wavelength of 1cm to 1m (frequencies of 30GHz to 300MHz respectively used for RADAR transmission and the remaining wavelength range is used for telecommunication. In order not to interfere with these uses, domestic and industrial microwave heaters are required to operate at either 12.2 cm (2.45 GHz) or 33.3cm (900MHz) unless the apparatus is shielded in such a way that no radiation losses occur. Laboratory/domestic microwave ovens generally operate at 2.45 GHz.
Microwave-assisted digestion:

Microwave system is designed with time-controlled programmable power supply, having a corrosion resistant, well-ventilated cavity and having all electronics protected against corrosion for safe operation. Use a unit having a rotating turntable with a minimum speed of 3 rpm to ensure homogeneous distribution of microwave radiation. Only laboratory-grade microwave equipment and closed digestion containers with pressure release that are specifically designed for hot and acid may be used.

Programme 1					
Step	**Time**	**Power(walt)**	**Pressure(bar)**	**Int. Temp. (°C)**	**Ext. Temp. (°C)**
1	00:05:00	250	3	150	70
2	00:50:00	400	6	180	80
3	00:04:00	500	10	210	80
Ventilation	00:30:00				

Open vessel and closed vessel microwave system:

Microwave sample preparation is now a standard analytical tool employing a variety of microwave equipment, including both low to high pressure closed vessels and atmospheric-pressure open-vessels. Both open vessels and closed-vessel microwave systems use direct absorption of microwave radiation through essentially microwave transparent vessel materials. Atmospheric pressure microwave systems can generate more stable temperature condition. In comparison, closed vessel microwave dissolution systems are limited by the temperature and pressure safety tolerances of the reaction vessel and the microwave absorption characteristics of the solution.

Microwave digestion rotor (MDR) technology provides analysts with unsurpassed performance capabilities and the highest standard of safety in closed vessel microwave digestion. The rotor consists of high-density polypropylene core. The compact core has the required strength to withstand the extreme pressure generated inside the vessel during digestion. Special plastic compression screws are located on the top of the rotor next to each niche. The vessel cover is also made of PFA (Teflon).

Microwave digestion of organic samples frequently involves exothermic reactions, which instantaneously generate large amount of decomposition gases (CO_2 and NO_x) as the sample is oxidised. The relief valve is capable of instantaneously venting excess decomposition gases and resealing the vessel for completion of the digestion procedure.

The acid used in microwave digestion may be classified in two main groups:

- Non-oxidising acids such as hydrochloric acid, hydrofluoric acid, phosphoric acid, dilute sulphuric acid and perchloric acid
- Oxidising acids, such as nitric acid, hot concentrated perchloric acid, concentrated sulphuric acid and hydrogen peroxide

Zinc (Zn)

28.12

28.12.1 Scope and application

Zinc is an essential and beneficial element for human growth. Concentration above 5mg/L can cause a bitter astringent taste in water. The zinc concentration in water varies from 0.06 to 7.0 mg/L with a mean concentration of 1.33 mg/L. Zinc most commonly enters the domestic water supply from deterioration of galvanized iron and dezincification of brass. In such cases lead and cadmium also may be present because they are impurities of the zinc used in galvanising. Zinc in water also may result from industrial waste pollution. The FAO recommended maximum level for zinc in irrigation water is 2mg/L. The US EPA primary drinking water standard MCL is 5 mg/L.

Methods for analysis:

A. Inductively coupled plasma method/AAS

B. Zincon Method

A. Inductively coupled plasma method

Please refer section 28.6 A.

B. Zincon method

28.12.2 Summary of the method

Principle: Zinc forms a blue complex with 2carboxy-2'-hydroxy-5-sulphoformazyl benzene (Zincon) in a solution buffered to pH 9.0. Other heavy metals likewise form coloured complexes with Zincon. Cyanide is added to complex zinc and heavy metals. Cyclohexane is added to free zinc selectively from its cyanide complex so that it can be complexed with Zincon to form a blue colour. Sodium ascorbate reduces manganese interference. The developed colour is stable except in the presence of copper.

Interference: The following ions interfere at concentrations exceeding those listed:

Ion	mg/L	Ion	mg/L
Cd^{2+}	1	Cr^{3+}	10
Al^{3+}	5	Ni^{2+}	20
Mn^{2+}	5	Cu^{2+}	30
Fe^{3+}	7	Co^{2+}	30
Fe^{2+}	9	CrO_4^{2-}	50

Minimum detectable concentration: 0.02 mg Zn/L

28.12.3 Apparatus and equipment

i. Colorimetric equipment: One of the following is required:

ii. Spectrophotometer, for measurements at 620 nm, providing a light path of 1 cm or longer.

Filter photometer, providing a light path of 1 cm or longer and equipped with a red filter having maximum transmittance near 620 nm. Deviation from Beer's Law occurs when the filter ban pass exceed 20 nm

iii. Graduated cylinders, 50 mL, with ground glass stoppers, Class B or better.

iv. Erlenmeyer flasks, 50mL.

v. Filtration apparatus: 0.45µm filters and filter holders.

28.12.4 Reagents and standards

i. Metal-free water: Use water for rinsing apparatus and preparing solutions and dilutions.

ii. Stock zinc solution: Dissolve 1000 mg (1g) zinc metal in 10mL 1 + 1HNO_3. Dilute and boil to expel oxides of nitrogen. Dilute to 1000 mL; 1mL = 1mg Zn.

iii. Standard zinc solution: Dilute 10mL stock zinc solution to 100 mL with water; 1 mL =10µg Zn.

iv. Solution ascorbate, fine granular powder, USP.

v. Potassium cyanide solution: Dissolve 1 g KCN in approximately 50 mL water and dilute to 100 mL.

Caution: potassium cyanide is a deadly poison. Avoid skin contact or inhalation of vapour. Do not pipette by mouth or bring in contact with acids.

vi. Buffer solution: pHn9.0: Dissolve 8.4g NaOH pellets in about 500 mL water. Add 31 g H3BO3 and swirl or stir to dissolve. Dilute to 1000mL with water and mix thoroughly.

vii. Zincon reagent: Dissolve 100 g Zincon (2-carboxy-2f-hydroxy-5'-sulfoformazyl benzene) in 100 mL methanol. Because Zincon dissolves slowly, stir and /or let stand overnight.

viii. Cyclohexanone: purified

ix. Hydrochloric acid, HCl, conc. and 1N.

x. Sodium hydroxide, NaOH, 6N and 1N.

28.12.5 Procedure

Preparation of Colorimetric standards: Accurately deliver 0, 0.5, 1.0, 3.0, 5.0, 10.0, 14.0 mL standard zinc solution to a series of 50 mL graduated mixing cylinders. Dilute each to 20 mL

to yield solutions containing 0, 0.25, 0.5, 1.5, 2.5, 5.0 and 7.0 mg Zn/L respectively.

Add the following to each solution in sequence, mixing thoroughly after each addition: 0.5 g sodium ascorbate, 5 mL buffer solution, 2 mL KCN solution and 3.0 mL Zincon solution. Pipette 20 mL of solution in a clean 50 mL Erlenmeyer flask.

Add 1 mL Cyclohexanone to the Erlenmeyer flask. Swirl for 10s and note the time. Transfer portions of both the solutions to clean sample cells. Use solution without Cyclohexanone to zero colorimeter. Read and record absorbance for solution with cyclohexanone after one minute. The calibration curve does not pass through zero because of the colour enhancement effect of Cyclohexanone on zincon.

Treatment of samples: To determine readily acid extractable total zinc, add 1 mL conc. HCl to 50 mL sample and mix thoroughly. Filter and adjust to pH 7. To determine dissolved zinc, filter sample through a 0.45µm embrane filter. Adjust to pH 7 with 1N NaOH or 1N HCl if necessary after filtering.

Sample analysis: Cool samples to less than 30°C if necessary. Analyse 20 mL of prepared sample. If the zinc conc. exceeds 7 mg Zn/L, prepare a sample dilution and analyse 20 mL portion.

Zinc µg	Colour
0	Green
1	Blue
2	Blue-violet
3	Violet
4	Red-violet
5	Red-violet

28.12.6 Calculation

Read zinc concentration (in mg/L) directly from the calibration curve.

28.12.7 Bibliography

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29 CYANIDE

Introduction:

Cyanide refers to all of the CN groups in cyanide compounds that can be determined as the cyanide ion, CN^-; by the methods used. The cyanide compounds in which cyanide can be obtained as CN^- are classed as simple and complex cyanide.

Colorimetric Method

29.1 Principle

This method is used for the Colorimetric determination of cyanide in water. The absorbance of the red blue dye developed is measured at 578mm. The intensity of the colour depends upon the concentration of cyanide.

29.2 Equipment

Colorimetric equipment: One of the following is required

a. Spectrophotometer, for use at 578mm, providing a light path of 1cm.
b. Filter photometer, providing a light path of 1cm and equipped with a red filter having maximum transmittance at 570 to 50nm.

29.3 Reagent and standards

a. Chloramines-T solution: Dissolve 1.0g white, water-soluble powder in 100mL water. Prepare weekly and store in refrigerator.
b. Stock cyanide solution: Dissolve approximately 2 g KOH and 2.51g KCN in 1 L distilled water.
c. Standard cyanide solution: dilute a calculated volume (Approximately 10mL) of the stock KNC solution based on the determined concentration to 1000mL with 0.25N NaOH. Prepare this solution fresh daily and keep in a glass stoppered bottle.
d. Pyridine-barbituric acid reagent: Place 15g barbituric acid in a 250mL volumetric flask and add just enough water to wash the sides of the flask and wet the barbituric

acid. Add 75mL pyridine and mix. Add 15mL conc. HCl, mix and cool to room temperature. Dilute to the mark with water and mix.

e. Sodium dihydrogen phosphate 1M: Dissolve 138g $NaH_2PO_4.H_2O$ in 1L distilled water, refrigerate.

f. Sodium hydroxide solution, 0.25N: Dissolve 10g NaOH in 1L distilled water.

29.4 Sample collection, preservation and storage

Because most cyanide is very reactive and unstable, analyse samples as soon as possible. If the sample cannot be analysed immediately, add NaOH pellets or a strong NaOH solution to raise the pH of the sample to 12 or above and store in a closed, dark bottle in a cool place.

To analyse for cyanogens chloride, collect a separate sample and omit the NaOH addition because cyanogens chloride is converted rapidly to cyanate at high pH. Make colorimetric estimation immediately after sampling.

Oxidising agents such as chlorine decompose most of the cyanides. Test 2 drops of sample with 2 drops of ortho-tolidine reagent on a spot plate. Yellow to orange colour indicates the need for treatment. Add ascorbic acid, a few crystals at a time, until 2 drops of sample produce no colour with the spot test. Then add an additional 0.06g of ascorbic acid for each L of sample. If possible, carry out this reducing procedure before preserving sample as described in the preceding paragraph. Sulphide in the sample will convert CN- to SCN- rapidly, especially at high pH. Test for sulphides if present; remove before raising the pH to stabilise the sample.

29.5 Procedure

The nature of the preliminary treatment will vary according to the interfering substances present. Sulphides, fatty acids and oxidizing agents are removed by special procedures. Most other interfering substances are removed by distillation. The importance of the distillation procedure cannot be over emphasised.

Take a portion of the absorption liquid obtained after distillation, such that the CN concentration falls in the measurable range (0.2µg in 20mL) and dilute to 20mL with 0.25N NaOH. Place the portion in a 50mL volumetric flask. Add 15mL phosphate buffer thoroughly. Add 2.0mL chloramines-T solution and swirl to mix. Immediately add 5mL pyridine-barbituric acid solution and again swirl to mix. Dilute to the mark with the water; mix well in inversion. Allow 8 min for colour development.

Measure the absorbance of the developed colour with the photometer at 578nm in a 1.0cm cell within 15min for the time at which the pyridine barbituric acid reagent is added. Using the calibration curve and the formula, determine the cyanide concentration in the original sample.

29.6 Calculation

CN, mg/L = A x B / C x D
Where,

A = µg CN read from calibration curve,

B = total mL absorbing solution used in the distillation,

C = mL original sample used in the distillation and

D = mL absorbing solution used.

29.7 Calibration

From the standard prepare a blank and a series of standards containing from 0.2 to 6µg CN in 20mL solution using 0.25N NaOH for all dilutions. Treat the standards as per standard. Plot the absorbance of the standards against CN concentration in micrograms. On the basis of the first calibration curve, prepare additional standards containing less than 0.2 and more than 6µg of cyanide to determine the limits measurable with the photometer being used.

29.8 Precision and Bias

The analysis of a mixed cyanide solution containing sodium, zinc, copper and silver cyanides in tap water gave a precision within the designated range as follows:

$$S_T = 0.115\ X + 0.031$$

Where S_T = overall precision in mg/L and X = concentration of cyanide in mg/L.

The recovery of the cyanide should be determined by spiking the cyanide standard in water sample and same procedure need be followed for specified sample as normal samples. The analysis should be done in duplicate.

29.9 Pollution prevention and waste management

The toxic effects of even low concentrations of cyanide on aquatic life and on the biota of wastewater generated after analysis should be disposed off safely.

CPCB brings out Reports under the CUPS series :

CUPS/45/99-2000	Sewage Management in Trans-Yamuna Region of Delhi : Status and Needs
CUPS/46/99-2000	Status of Solid Waste Generation, Collection, Treatment and Disposal in Metrocities
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