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EXPERIMENT 01

OBJECTIVE:

To prepare a buffer solution and to determine p^H of that buffer solution and other samples.

APPARATUS REQUIRED:

1. p^H meter
2. Beaker (250ml)
3. Glass rod
4. Test tubes
5. Pipette (1ml)

CHEMICALS REQUIRED:

1. Solutions of glacial acetic acid.
2. Sodium acetate
3. Hydrochloric acid.

The experiment comprises of 2 parts, such as :

- (i) Preparation of Buffer Solution.
- (ii) Determination of their p^H .

(I) PREPARATION OF BUFFER SOLUTION :

THEORY: The resistance to change hydrogen ion concentration of a solution on addition of acid or alkali is known as Buffer action. The buffer solution is variably consists of a mixture of a weak acid or weak base and its salt. The combination of weak

acid, weak base and its salt suppress the dissociation of acid or base due to common ion effect so that the hydrogen ion or hydroxyl ion availability is lower than in the pure solution at a comparable concentration.

(II) DETERMINATION OF p^H OF BUFFER SOLUTION :

THEORY:

The ionic product of water and hydrogen ion concentration are related from the dissociation of water molecule as follows.



$$K_a = [H^+] [OH^-] = 10^{-14}$$

$$\text{At neutral point, } [H^+] = [OH^-] = 10^{-7}$$

The no. of gram ions of hydrogen present in 1 lit of solution is known as hydrogen ion concentration. If hydrogen ion concentration is more than 10^{-7} then the solution is acidic, if it is less than 10^{-7} then it is basic. Sorenson (1909) introduced the term p^H and suggested its use to express the acidity or alkalinity of solution which is defined as the negative logarithm of the hydrogen ion concentration.

$$p^H = -\log [H^+]$$

$$\text{For pure water, } p^H = -\log [H^+] = -\log 10[10^{-7}] = 7$$

$$\text{For acidic solution, } p^H < 7$$

$$\text{For basic solution } p^H > 7$$

Tabulation:

No.of Obs.	Vol.of 0.2M Acetic acid in ml (V1)	Vol.of 0.2M Sodium acetate in ml (V2)	pH Calculated	pH Observed	Remarks
1					
2					
3					
4					

Calculation:

EXPERIMENT 02

OBJECTIVE:

Determination of total hardness of water by EDTA method.

Apparatus required : Burette (50 ml), Pipette (10ml), Measuring cylinder (10ml), conical flask (100 ml)

Reagents required :

1. Solution of Ethylenediamine tetraacetic acid (EDTA)
2. Ammonia buffer solution
3. Erichrome black T(EBT)
4. Standard hard water solution.

Theory:

Hardness of water can be defined as that property of water, which prevents the lathering of soap. Hardness occurs mainly due to the presence of calcium and magnesium ions in the water. Heavy metal ions, and higher valent cations when present in water also cause hardness but to a lesser degree. Hence, in practice the hardness of water is taken as a measure of its Ca^{2+} and Mg^{2+} content. Mono-valent cations of alkali metals never produce hardness. Total hardness is due to the summation of permanent & temporary hardness. The Total hardness of water can be determined by complexo-metric titration. EDTA used as complexing agent & Eriochrome Black T (EBT) as indicator at a p^{H} of 9—10.

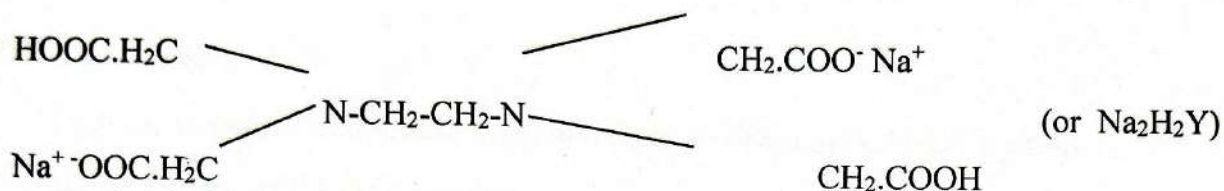
Unit of hardness:

Hardness of water is measured in **parts per million** (ppm) (w/v). All the hardness causing ions are expressed in terms of their respective weights equivalent to CaCO_3 .

$$1\text{ppm} \equiv 1\text{mg} / \text{L} \equiv 1\text{g} / 10^6 \text{ml}$$

Ethylene diamine tetraacetic acid (EDTA solution)

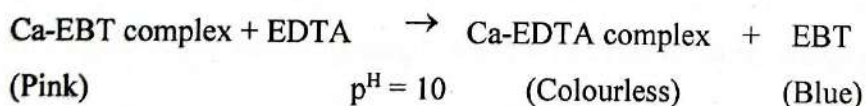
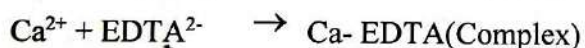
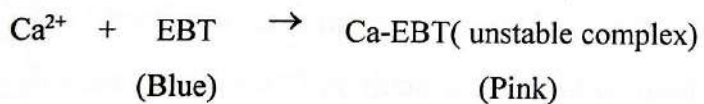
EDTA is a complexing agent (a remarkable ligand) which forms stable soluble complex with Ca & Mg ions having the following structure:



The titration of EDTA with Ca or Mg ions is a 1:1 ratio complex formation of cage like structure. The equilibrium is affected by the pH of the medium ($\text{pH } 9\text{--}10$).

Indicator:

Eriochrome Black –T is an organic colouring substance that undergoes changes in colour while forming a less stable complex with Ca or Mg ions (compared to Ca or Mg-EDTA complex). Total reaction with colour changes may be represented as follows;



Similar reaction takes place for Mg^{2+} ion.

Buffer solution:

The colour changes of the indicator are sensitive to pH of the medium. Hence to maintain the pH to appropriate range (9-10), ammonia buffer solution ($\text{NH}_4\text{Cl} + \text{NH}_4\text{OH}$) is added. At this pH of the medium, the complex between metal ions and EDTA is also stable.

PROCEDURE:

- Wash the burette with distilled water and rinse it with supplied EDTA solution.
- Fill the burette with EDTA solution.
- Remove air bubbles, if any and adjust to a level correctly.
- Fix the loaded burette to the burette stand.
- Wash & rinse the pipette with distilled water.
- Take 10 ml of the standard hard water with pipette into a 100ml conical flask previously washed with distilled water.
- To it, add 3 ml of ammonia buffer solution ($\text{pH} \approx 10$) with measuring cylinder.
- Add 4 drops of the indicator {Eriochrome black-T (EBT)} solution & note the wine red colour.
- Titrate with EDTA solution of known strength until the colour turns sharply from wine red to blue indicating the end-point.
- Note the initial and final burette readings and enter it in the table-I.
- The titration process may be repeated to get the concordant reading.
- Similar titration will be done with supplied hard water and the values will be written in Table-II.

Observation:

In this titration the colour changes from **wine red to blue** sharply at the end-point

Tabulation-I:

No. of Obs.	Vol. of standard hard water in ml	I.B.R. in ml	F.B.R. in ml	Diff. in ml	Volume of EDTA = X in ml
1					
2					
3					
4					

Tabulation-II:

No. of Obs	Vol. of hard water in ml	I.B.R. in ml	F.B.R. in ml	Diff. in ml	Volume of EDTA = Y in ml
1					
2					
3					
4					

Calculation:

X= ml , Y= ml

$$\frac{X = \text{amount of EDTA with SHW}}{Y = \text{amount of EDTA with hard water}} = \frac{\text{Known hardness in ppm}}{\text{Unknown hardness in ppm}}$$

Hence unknown hardness = $(Y/X) \times \text{known hardness}$ =
ppm.

Conclusion:

The total hardness of the given water sample is found to be ppm(w/v) as CaCO_3 .

Precautions:

- i) Parallax error should be avoided while taking the burette reading.
- ii) The titrant should be added drop wise drop towards the end.

EXPERIMENT 03

OBJECTIVE:

To determine the acidity of water supply

APPARATUS REQUIRED:

1. Burette
2. Conical flask
3. Funnel

CHEMICALS REQUIRED:

1. Standard NaOH (N/50)
2. Phenolphthalein
3. Sodium thiosulphate ($\text{Na}_2\text{S}_2\text{O}_3$)
4. Tap water.

THEORY:

Measurement of acidity is important as acidic water are corrosive and corrosion producing substances have to be controlled or removed. If the acidic waters are used for steam generation in boilers, it results in corrosion & decreased efficiency of boiler. For mineral acids, the titration is carried to a p^{H} of about 4.5 by using methyl orange indicator, giving a colour change from red to yellow. The acidity thus determined is called methyl orange acidity. Total acidity or phenolphthalein in acidity is determined by carrying the titration to phenolphthalein end point of 8.3 (measuring mineral acids, organic acids and free CO_2). The results are expressed as part of equivalent CaCO_3 per million parts of water.

END POINT:

1. Appearance of pink colour with NaOH in the burette and a drop of phenolphthalein.
2. Change of yellow colour to pink with methyl orange as an indicator.

PROCEDURE:

1. Pipette out 100ml of the water sample into a conical flask.
2. Add 1 drop of N/10 $\text{Na}_2\text{S}_2\text{O}_2$ solution to destroy any residual chlorine
3. Add 2-3 drops of methyl orange indicator and titrate against N/50 NaOH solution until the red colour changes to yellow.
4. Take at least 3 concordant readings and record the volume of NaOH (Sodium hydroxide) used as Y ml.

TABULATION:

No. of obs	Vol. of water in ml	IBR in ml	FBR in ml	Difference in ml	Remark
1					
2					
3					
4					

CALCULATION:

Volume of water sample taken for each titration = 100

Volume of N/50 NaOH used in presence of methyl orange indicator = Y ml

Volume of N/10 NaOH used in presence of Phenolphthalein indicator = Z ml

PHENOLPHTHALEIN ACIDITY:

$$N_1 V_1 = N_2 V_2$$

Sample = N/50 NaOH

$$N_1 \times 100 = \frac{1}{50} \times Y$$

$$\rightarrow N = \frac{Y}{50 \times 100}$$

Phenolphthalein acidity =

PHENOLPHTHALEIN ACIDITY:

1. Proceed as above 2-3 drops of Phenolphthalein indicator (in place of methyl orange) and titrate the sample against N/50 NaOH (from burette) until the solution is turned to pink colour and the colour persists for at least 30 second.
2. Repeat the titration to get atleast two concordant readings.
3. Record the volume of NaOH used as Z ml.

OBSERVATION:

In this titration, the colour changes from colourless to light pink.

CONCLUSION:

The acidity of the given water sample is found to be ppm.

PRECAUTION:

1. The cleaned apparatus should be used for the titration.
2. To avoid loss of CO_2 , the titration should be carried out quickly & vigorous shaking should be avoided.

EXPERIMENT 04

OBJECTIVE:

To determine the alkalinity of a given water sample.

APPARATUS REQUIRED:

1. Burette
2. Pipette
3. Conical flask
4. Glazed tile

CHEMICALS REQUIRED:

1. N/10 HCL Solution
2. Phenolphthalein indicator
3. Methyl orange indicator.

THEORY:

Alkalinity of water, which is a measure of the ability of water to neutralize the acids, is due to presence of bicarbonates, carbonates & hydroxides of Ca & Mg. Determination of alkalinity due to different ions is based on the titration of the water sample against a standard acid making selective use of indicators. The indicators used are phenolphthalein & methyl orange.

The reaction taking place is as follows:

1. $\text{OH}^- + \text{H}^+ \longrightarrow \text{H}_2\text{O}$
2. $\text{CO}_3^{2-} + \text{H}^+ \longrightarrow \text{HCO}_3^-$



A known volume of the sample is titrated against a standard acid a sign phenolphthalein as indicator to phenolphthalein end point [P] & continuing the titration during methyl orange as indicator point [M]. The volume of acid ran down upto phenolphthalein end point [P] corresponds to the completion of the equation (1) & (2) given above, while the volume of acid ran down after [P] corresponds to the completion of equation (3). The total amount of acid used from the beginning of the experiment, i.e. $\rightarrow$ [M] corresponds to the completion of the reaction (1) to (3).

The result may be summarised in the following table from which the alkalinity due to different ions can be calculated.

ALKALINITY DUE TO DIFFERENT IONS:

Titration result	OH^-	CO_3^{2-}	HCO_3^-
[P] = 0 [P] = [M]	NIL [P] or [M]	NIL NIL	[M] NIL
$[P] = \frac{1}{2} [M]$	NIL	2 [P] or [M]	NIL
$[P] > \frac{1}{2} [M]$	2 [P] - [M]	2 { [M] - [P] }	NIL
$P < \frac{1}{2} [M]$	NIL	2 [P]	[M] - 2 [P]

Alkalinity is generally expressed as parts per million in terms of CaCO_3

PROCEDURE:

1. Take 50ml of water sample in a 250ml conical flask.
2. Add 2-3 drops of phenolphthalein indicator.
3. The colour of the solution will become pink.
4. Now titrate this solution against 0.1 N HCL taken in a burette till colour of the solution disappears.

5. It shows all the carbonates have been converted in to bio-carbonates.
6. Note the titrate value of the phenolphthalein and point [P].
7. Add 2-3 drops of Methyl orange indicator to the same solution and continue the titration until the sharp colour changes from yellow to rose red takes place.
8. Note the total titre value from the beginning of the experiment as methyl orange end point [M].

TABULATION:

No. of obs	Volume of water	IBR	Titration with phenolphthalein		Titration with methyle orange.	
			FBR	Volume of HCL used [P] = FBR-IBR	FBR	[M]=FBR-IBR
1						
2						
3						

CALCULATION:

OBSERVATION:

In this titration, when phenolphthalein is used as indicator the colour changes from light pink to colourless & when methyl orange is used as indicator the colour changes from yellow to rose red colour.

CONCLUSION:

The supply water sample contains

Total alkalinity =

Alkalinity w.r.t CO_3^{2-} =

Alkalinity w.r.t HCO_3^- =

Alkalinity w.r.t OH^- =

EXPERIMENT 05

OBJECTIVE:

To determine dissolved oxygen in a sample of water.

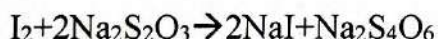
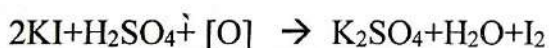
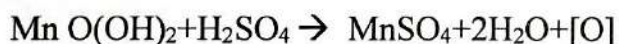
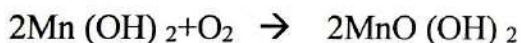
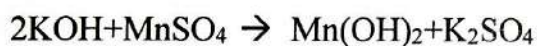
Apparatus required: Burette, pipette, conical flask, beaker, measuring cylinder

Chemicals required: MnSO_4 , KOH , KI , $\text{Na}_2\text{S}_2\text{O}_3$, Starch, and NaN_3

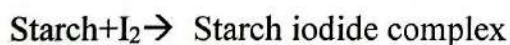
Theory:

It is based on oxidation of potassium iodide. The liberated iodine is titrated against standard hypo solution using starch as a final indicator. Since oxygen in water is in molecular state and not capable to react with KI , an oxygen carrier manganese hydroxide is used to bring about the reaction between KI and O_2 . Manganous hydroxide is produced by the action of potassium hydroxide and manganous sulphate.

Chemical reaction:



Sodium tetrathionate



(Blue in colour)

Procedure:

1. Take 500ml of water in a D.O bottle.
2. Add 10ml of alkaline KI and 10 ml of MnSO_4 into it.

3. Stopper the bottle and shake it well.
4. Keep the bottle in dark for 5 min and add conc H_2SO_4 till the brown precipitates are dissolved.
5. Take 100 ml of the above solution in a conical flask. Titrate against hypo till the color changes to light Yellow.
6. Add 3-4 drops of starch in to it and the color changes to blue.
7. The blue color solution is titrated against hypo solution till blue color disappeared.
8. This is end point of the titration. Repeat this process till to get three concordant reading.

Tabulation:

No of obs	Volume of water in ml	IBR in ml	FBR in ml	Diff in ml	Remark
1					
2					
3					
4					

Calculation:

1000 ml of 1N $\text{Na}_2\text{S}_2\text{O}_3$ = 8 gm of O_2

$$\text{"V" ml of N/40 } \text{Na}_2\text{S}_2\text{O}_3 = \frac{8V}{40 \times 1000} \text{ gm of } \text{O}_2 = \frac{8V' \times 1000}{40 \times 1000} \text{ mg of } \text{O}_2 = \frac{V}{5} \text{ mg of } \text{O}_2.$$

100 ml of water sample contain = $V/5$ mg of O_2

1 lit of water sample contains = $2V$ mg of O_2 = ppm

Conclusion:

The amount of dissolved oxygen in a sample of water is found to be ppm.

EXPERIMENT 00

OBJECTIVE:

To determine the optimum dose of coagulants for a water sample by jar test.

APPARATUS REQUIRED:

1. Jar testing Equipment with multiple spindle stirrer

Preparing Coagulant and Flocculent Solutions for Jar Testing:

For existing applications, determine the current feed rate of the coagulant and flocculants, if available. Additionally, if the coagulant is being diluted to a certain percent solution, it would be beneficial to know this information as well. Generally speaking, coagulant chemistries are fed neat and are not made down into solutions. Determine the flocculent solution prepared. Emulsion polymers require strong sheer force mixing and some age time to be inverted to full activity. Again, determine what the current make-down solution is for the existing application and dosage rate. Base the dosage of the flocculants aid on product dosage, not the make-down solution dosage. Make-down solution dosage for flocculants is normally 0.1 – 1% solutions.

A. Coagulant Solutions

Prepare your coagulant solution based on the information generated in item A and the size of the beakers you plan to use in your jar tests. For example, if your coagulant dosage is within the range of 5 to 100 ppm, you may want to make a 1% solution of the coagulant to add to your 1,000 ml beakers. This solution strength would result in you adding approximately 0.5 mls – 10 mls of your 1% solution to 1000 mls sample water. However, if your coagulant dosage is several hundred parts per million or greater, it would be better to make a 5 of 10% solution to be added to your 1,000 ml beakers.

Making a 1% Solution of a Coagulant with a Specific Gravity of 1.2

1. Determine the amount of solution to be made (200 mls).
2. Amount of coagulant product needed = mls of 1% solution to be made, multiplied by 1%, and divided by specific gravity of coagulant = $200 \times .01 \div 1.2 = 1.67$ mls. (2 grams)
3. Place amount of coagulant needed into approximately 198 mls (198 grams) of distilled water, for a 1% solution.

Note: If 10% solution is made, 16.7 mls (20 grams) of coagulant would be placed into 180 mls (180 grams) of distilled water.

4. The solution can be mixed by placing it in a clean sample bottle, securing a lid, and shaking it vigorously for 30 seconds to 1 minute.

B. Flocculent Solutions

Make the appropriate percent solution for the flocculants to be tested and the feed equipment to be used. If you cannot determine this information, a good starting point is a 0.1% solution. The calculations for determining the amount of emulsion flocculent product to use are the same as for coagulant; however, the mixing procedure requires much more agitation and mixing.

Making an Emulsion Flocculent Solution:

1. Determine the amount of solution to be made (100 mls).
2. Amount of flocculent product needed = number of mls of solution to be made, multiplied by the percent solution to be made, divided by the specific gravity of the flocculent = $100 \times 0.001 \div 1.05 =$ approximately 0.1 mls (0.1 grams) of flocculent product.
3. Amount of water for solution = 100 grams – 0.1 gram flocculent product = 99.9 grams (99.9 mls) of water.
4. Place 99.9 mls of water into a plastic 200 ml mix cup.

5. Using a Braun mixer (or similar), begin mixing the distilled water in the mix cup. A stir rate of 800-1000 rpm is desired.
6. Shake the emulsion sample for 5-10 seconds until the product is uniform.
7. Immediately add 0.1 mls of flocculent product into the vortex area of the water as it is mixing.
8. Mix for a total of approximately 1 minute while turning the mixer section from one side to the other side of the mix cup for approximately 20 second intervals.
9. Discontinue mixing. Allow emulsion flocculent mixture to drain from the Braun mixer into the cup and clean the mixer with a dry paper towel.
10. Ideally, turbulent mixing should continue for 30 minutes for most efficient unwinding of the polymer. At least 30 minutes should be provided with occasional mixing.

Note: If a mixer is not available, invert the emulsion polymer as follows. (This method will yield less efficient inversion and unwinding of the polymer).

- a. Use a container with a lid. Measure the water out and add it to the container.
- b. Rapidly shoot the emulsion into the water using a syringe. With this method, target a 0.1% solution.
- c. Immediately cap the container and vigorously shake for 1 minute.
- d. Provide additional occasional moderate shaking for 30 minutes. The resulting solution should be white, translucent to opaque, and homogenous.

Making a Flocculent Solution with Dry Polymers:

1. Determine the amount of solution to be made (100 mls).
2. Generally, 0.1% solution strength is a good amount, so weigh out 0.1 grams and add to 100 mls.

(If you don't have access to a metric balance, you can come close by using the brass dippers in the test kits as holding 0.1 grams of flocculent. A 0.1 gram plastic dipper will provide about 0.06 grams of dry flocculent. This will vary somewhat by the flocculent being used).

3. Slowly add the flocculent particles into the vortex of water, mixing at 800-1000 rpm, then offset and continue turbulent mixing for 30 minutes. Alternatively, add slowly to a container, cap it, and vigorously shake for 1 minute, then provide additional moderate shaking for 30 minutes.

The resulting solution should be clear and homogenous. If fisheyes or globs are present, continue additional mixing.

Testing

Using existing plant operating data, determine the range of coagulant dosage and flocculent dosage you would like to evaluate in your jar test. In the first iterations of jar tests, you will probably be changing the coagulant dosages while maintaining a constant flocculent dosage. The proper coagulant chemistry and dosage is what you want to determine first. Sometimes, you may not even apply flocculent until you have correctly identified the most effective coagulant chemistry.

Example: Plant is currently feeding a coagulant at 60 ppm and flocculent at 1 ppm. You have a 4-beaker gang stirrer and plan to dose the coagulants in the jars at 20 ppm increments, starting with 20 ppm. You will dose all the jars with 1 ppm of flocculent. Calculate the amount of 1% solution of coagulant added to 1,000 mls of water to achieve a dosage of 20 ppm.

Determine what 1 ml of a 1% solution into 1,000 mls sample is equal to in mg/L or ppm.

1 ml of a 1% solution = 0.01 grams.

Divide by 1,000 mls and 1 ml in 1000 mls = 0.00001 g/L.

Multiply by 1,000,000 to get mg/L which is 10 mg/L (ppm) or a

1% solution = 10,000 ppm. Dilute a 1 ml 1% solution with 1,000 mls of water to be tested. $10,000 \div 1,000 = 10$ ppm.

When using a 1% solution of coagulant and 1,000 ml jars, 1 ml of solution added = 10 ppm. For a 500 ml sample, 1 ml of a 1% solution = 20 ppm.

Therefore, for jar 1, you will add 2 mls, add 4 mls to jar 2, 6 mls to jar 3, and 8 mls to jar 4 to have respectively 20, 40, 60, and 80 ppm of coagulant.

Determine the amount of 0.1% flocculent solution added to 1,000 ml jars to achieve 1 ppm dosage.

$1 \text{ ppm} = (X \text{ mls of } 0.1\% \text{ solution multiplied by } 0.001 \text{ divided by } 1,000 \text{ mls of sample}) \text{ multiplied by } 1,000,000.$

$X = 1 \text{ ml of } 0.1\% \text{ flocculent solution, therefore, we are going to add } 1 \text{ ml of the } 0.1\% \text{ flocculent solution to achieve } 1 \text{ ppm dosage to all four beakers.}$

Results:

EXPERIMENT 07

OBJECTIVE:

To perform microbiological culture analysis of bacterial samples followed by MPN (*Most Probable Number*) test.

PRINCIPLE

The bacteriological examination of water is performed routinely by water utilities and many governmental agencies to ensure a safe supply of water for drinking, bathing, swimming and other domestic and industrial uses. The examination is intended to identify water sources which have been contaminated with potential disease-causing microorganisms. Such contamination generally occurs either directly by human or animal feces, or indirectly through improperly treated sewage or improperly functioning sewage treatment systems. The organisms of prime concern are the intestinal pathogens, particularly those that cause typhoid fever and bacillary dysentery.

Since human fecal pathogens vary in kind (viruses, bacteria, protozoa) and in number, it would be impossible to test each water sample for each pathogen. Instead, it is much easier to test for the presence of nonpathogenic intestinal organisms such as *E. coli*. *E. coli* is a normal inhabitant of the intestinal tract and is not normally found in fresh water. Therefore, if it is detected in water, it can be assumed that there has been fecal contamination of the water.

In order to determine whether water has been contaminated by fecal material, a series of tests are used to demonstrate the presence or absence of coliforms. The coliform group is comprised of Gram-negative, nonspore-forming, and aerobic to facultatively anaerobic rods, which ferment lactose to acid and gas. Two organisms in this group include *E. coli* and *Enterobacter aerogenes*; however, the only true fecal coliform is *E. coli*, which is found only in fecal material from warm-blooded animals. The presence of this organism in a water supply is evidence of recent fecal contamination and is sufficient to order the water supply closed until tests no longer detect *E. coli*.

In this exercise, you will be testing water samples for the presence of coliforms. There will be three principal tests: the presumptive, confirmed and completed tests (see flow-chart).

STANDARD WATER ANALYSIS

The Presumptive Test

In the presumptive test, a series of lactose broth tubes are inoculated with measured amounts of the water sample to be tested. The series of tubes may consist of three or four groups of three, five or more tubes. The more tubes utilized, the more sensitive the test. Gas production in any one of the tubes is presumptive evidence of the presence of coliforms. The most probable number (MPN) of coliforms in 100 ml of the water sample can be estimated by the number of positive tubes.

The Confirmed Test

If any of the tubes inoculated with the water sample produce gas, the water is presumed to be unsafe. However, it is possible that the formation of gas may not be due to the presence of coliforms. In order to confirm the presence of coliforms, it is necessary to inoculate EMB (eosin methylene blue) agar plates from a positive presumptive tube. The methylene blue in EMB agar inhibits Gram positive organisms and allows the Gram-negative coliforms to grow. Coliforms produce colonies with dark centers. *E. coli* and *E. aerogenes* can be distinguished from one another by the size and color of the colonies. *E. coli* colonies are small and have a green metallic sheen, whereas *E. aerogenes* forms large pinkish colonies.

If only *E. coli* or if both *E. coli* and *E. aerogenes* appear on the EMB plate, the test is considered positive. If only *E. aerogenes* appears on the EMB plate, the test is considered negative. The reasons for these interpretations are that, as previously stated, *E. coli* is an indicator of fecal contamination, since it is not normally found in water or soil, whereas *E. aerogenes* is widely distributed in nature outside of the intestinal tract.

The Completed Test

The completed test is made using the organisms which grew on the confirmed test media. These organisms are used to inoculate a nutrient agar slant and a tube of lactose broth. After 24 hours at 37°C, the lactose broth is checked for the production of gas, and a Gram stain is made from organisms on the nutrient agar slant. If the organism is a Gram-negative, non spore-forming rod and produces gas in the lactose tube, then it is positive that coliforms are present in the water sample.

FIRST PERIOD

Material:

1. Nine tubes of double-strength lactose broth
2. 10, 1.0 and 0.1 ml pipettes
3. Water samples

Procedure:

Presumptive Test

1. Take a water sample (dilute as instructed in some cases) and inoculate three tubes of lactose broth with 10 ml, three tubes with 1.0 ml and three tubes with 0.1 ml.
2. Incubate all tubes at 37°C for 24 hours.

SECOND PERIOD

Material:

1. EMB agar plates

Procedure:

Presumptive Test

1. Observe the number of tubes at each dilution that show gas production in 24 hrs.

Record results

2. Reincubate for an additional 24 hours at 37°C.

Confirmed Test

1. Inoculate an EMB plate with material from a tube containing gas.
2. Invert and incubate the plate at 37°C for 24 hours.

THIRD PERIOD

Material:

1. Lactose broth tubes
2. Nutrient agar slants

Procedure:

Presumptive Test

1. Observe the number of tubes at each dilution that show gas. Record results and determine the most probable number index.

Confirmed Test

1. Observe EMB agar plates. A positive confirmed test is indicated by small colonies with dark centers and a green metallic sheen (*E. coli*). Record results.

Completed Test

1. Inoculate a lactose broth tube and a nutrient agar slant with organisms from the EMB plate.
2. Incubate the broth tube and agar slant at 37°C for 24 hours.

FOURTH PERIOD

Procedure:

Completed Test

1. Check for gas production in the lactose broth tube.
2. Make a Gram stain from the organisms on the nutrient agar slant.
3. Record results.

Results:

EXPERIMENT 08

OBJECTIVE:

To determine the turbidity of different water samples.

APPARATUS REQUIRED:

1. Digital Turbidimeter

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 nephelometer 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 nephelometric turbidity units (NTU).

Sample collection, preservation and storage

Determine turbidity on the day the sample is taken. If 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.

Calibration

Prepare calibration curves in the range 0-400 units by carrying out appropriate dilutions of solutions and taking reading in turbidimeter.

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, analyze the sample in duplicate.

Results:

EXPERIMENT 09

OBJECTIVE:

To find out amount of total solids and dissolved solids in water sample.

Introduction

The term 'solid' refers to the matter either filterable or non-filterable that remains as residue upon evaporation and subsequent drying at a defined temperature. Further categorization 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 unfavorable 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 is important to decide upon the various unit operations and processes in physical and biological wastewater treatment and to assess its performance evaluation. For assessing compliance with regulatory agency, wastewater effluent limitations for various forms of solids act as indicating parameters.

A. Total solids

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 suspended 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 CO₂ volatilization of inorganic matter as much as consistent with complete oxidation of organic matter, are volatile solids.

Apparatus and equipment

- a. Electrically heated temperature controlled oven

- b. Monopan balance
- c. Evaporating dish (200mL)
- d. Pipettes
- e. Measuring cylinder (100mL)

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.

Calibration

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

Procedure

- a. Take a known volume of a well-mixed sample in a tarred dish ignited to constant weight (W1)
- b. Evaporate the sample to dryness at 103-105°C for 24hrs.
- c. Cool in desiccator, weigh and record the reading (W2)
- 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 (W3).
- f. The concentration is to be calculated in percent by weight.

Calculation

The total and the volatiles solids are expressed as:

$$\text{Total solids, mg/L} = (W2 - W1) \times 1000 / \text{mL of sample}$$

And

$$(W2 - W3) \times 1000 / \text{mL of sample}$$

Where W1, W2 and W3 are recorded in mg

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.

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

Sample collection, preservation and storage

Begin analysis as soon as possible due to impracticability of preservation of sample.

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 weight is obtained or weight loss is less than 0.5mg.

Calculation

$$\text{mg/l total filterable residue at } 180^{\circ}\text{C} = (A - B) \times 1000 / C$$

Where:

A = weight of dried residue + dish

B = weight of dish

C = ml of filtrate used

Results:

EXPERIMENT 004

OBJECTIVE:

To determine the Biological Oxygen Demand (BOD) of water sample.

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

Principle

This test measures the oxygen utilized 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 BOD₅ 20°C is established under same experimental condition (for example BOD₅, 27°C) is equivalent to BOD₃, 27°C) for Indian conditions. While reporting the results, the incubation period in days and temperature in °C is essential to be mentioned.

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.

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 $\text{Na}_2\text{HPO}_4 \cdot 7\text{H}_2\text{O}$ and 1.7g NH_4C ; in distilled water and dilute to 1000mL. The pH should be 7.2 without further adjustment. Discard reagent if there is any sign of biological growth.
- b. Magnesium sulphate: Dissolve 22.5g $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ 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 $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$ 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-(trichloromethyl) 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.

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 $\pm$ 3°C before analysis. State storage time and condition as part of results.

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 stabilize the water at 20°C/27°C.
- c. Add 1mL each of phosphate buffer, magnesium sulphate, and 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. Neutralize 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 $\text{Na}_2\text{S}_2\text{O}_3$ solution as described below.

c. Take 50mL of the sample and acidify with addition of 10mL 1 + 1 acetic acid. Add about 1g KI. Titrate with 0.025N $\text{Na}_2\text{S}_2\text{O}_3$, using starch indicator. Calculate the volume of $\text{Na}_2\text{S}_2\text{O}_3$ 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 $20 \pm 1^\circ\text{C}$ 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, $\text{DO} \geq 9\text{mg/L}$ 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 uptakes 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 3 days. See that the bottles have a water seal.
- c. Prepare a blank in triplicate by siphoning plain dilution water (without seed) to measure the O₂ consumption in dilution water.
- d. Also prepare a seed blank in triplicate to measure 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.

Calculations

Calculate BOD of the sample as follows:

- a. When dilution water is not seeded

$$\text{BOD as O}_2 \text{ mg/L} = (D_1 - D_2) \times 100 / \% \text{ dilution}$$

b. When dilution is seeded

$$\text{BOD O}_2 \text{ mg/L} = [(D_1 - D_2) - (B_1 - B_2)] \times 100 / \% \text{ dilution}$$

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

$$\text{BOD O}_2 \text{ mg/L} = [(D_1 - D_2) - (B_1' \times B_2')] \times F \times 100 / \% \text{ dilution}$$

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 = ratio 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.

Result:

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.

EXPERIMENT 11

OBJECTIVE:

To determine the Chemical Oxygen Demand (COD) of the water sample.

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 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 3-5 days 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.

Open Reflux method

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 oxidized 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. $2\text{K}_2\text{Cr}_2\text{O}_7 + 8\text{H}_2\text{SO}_4 \rightarrow 2\text{K}_2\text{SO}_4 + 2\text{Cr}_2(\text{SO}_4)_3 + 8\text{H}_2\text{O} + 3\text{O}_2$
- b. $\text{C}_6\text{H}_{12}\text{O}_6 + 6\text{O}_2 \rightarrow 6\text{CO}_2 + 6\text{H}_2\text{O}$
- c. $\text{Cr}_2\text{O}_7^{2-} + 6\text{Fe}^{2+} + 14\text{H}^+ \rightarrow 6\text{Fe}^{3+} + 2\text{Cr}^{3+} + 7\text{H}_2\text{O}$

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
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- k. Magnetic stirrer and stirring bars.

Reagents and standards

- a. Standard potassium dichromate solution, 0.25N (0.04167 M): Dissolve 12.259g $\text{K}_2\text{Cr}_2\text{O}_7$ dried at 103°C for 24h in distilled water and dilute to 1000mL. Add about 120 mg sulphamic acid to take care of 6 mg/L $\text{NO}_2 - \text{N}$.
- 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 $\text{Fe}(\text{NH}_4)_2(\text{SO}_4)_2 \cdot 6\text{H}_2\text{O}$ 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 $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$ 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 ($\text{HOOC.C}_6\text{H}_4.\text{COOK}$) in distilled water and dilute to 1000mL. This solution has a theoretical COD of $500\mu\text{g O}_2/\text{ml}$. This solution is stable when refrigerated, up to 3 months in the absence of visible biological growth.

Sample collection, preservation and storage

Preferably collect samples in glass bottles. Homogenize samples containing settleable solids. If there is delay in collection and analysis, preserve sample by acidification to $\text{pH} \leq 2$ using concentrated H_2SO_4 . Samples can be preserved for maximum 7 days.

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 standardization of ferrous ammonium sulphate, dilute 10ml standard $\text{K}_2\text{Cr}_2\text{O}_7$ 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.

Normality of FAS = $(\text{ml K}_2\text{Cr}_2\text{O}_7) (0.25) / \text{ml FAS required}$

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

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) $\text{K}_2\text{Cr}_2\text{O}_7$ 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 $\text{K}_2\text{Cr}_2\text{O}_7$ with 0.1M FAS using 2-3 drops of ferroin indicator. The sharp colour change from blue green to reddish brown indicates endpoint 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.

Calculations:

$COD \text{ as mg/L} = (a - b) \times N \times 8000 / \text{mL sample}$

Where, a = mL FAS used for blank

b = mL FAS used for sample

N = normality of FAS

8000 = Milieq. wt. of $O_2 \times 1000$

Result:

Precision and Bias

Precision and bias: A set of synthetic samples containing potassium hydrogen phthalate with a COD of 200mg/L was analyzed 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 handled very carefully to get accurate results

EXPERIMENT 12

OBJECTIVE:

To determine total residual chlorine in a water sample.

APPARATUS REQUIRED:

1. Burette
2. Pipette
3. Measuring flask
4. Watch glass
5. Weighing bottle
6. Glass rod

CHEMICALS REQUIRED:

1. Potassium iodide solution (10%)
2. Concentrated HCL.
3. Glacial Acetic Acid.
4. N/20 hypo solution ($\text{Na}_2\text{S}_2\text{O}_3$)
5. Starch solution (freshly prepared)

THEORY:

Living organism such as algae, fungi and bacteria are more abundant in surface drainage water, while in deep well water, the bacterial count is often low. The growth of these organisms in water used for industrial purpose may have serious consequences. The slime surrounding, these organisms causes them to add here to metal surface and the film of slime thus formed serves as a base to which suspended matter present in the water can add here. This leads to reduce the heat transfer rate and cause blockages in boiler. The growth of marine organisms such as mussels may lead to serious reduction in the carrying capacity of pipe lines and culverts. Thus measures to prevent the development

of living organisms are necessary. Organic growths generally take place most readily in water at temperature in the range 10-35 C.

PROCEDURE:

1. Transfer 50ml of a given water sample in a conical flask.
2. Add 10ml of KI solution and about 3ml of glacial acetic acid to maintain 3-4 p^H.
3. Cover the flask with stopper and mix the solution by shaking the flask.
4. After some time wash the sides of the flask with distilled water.
5. Titrate against standard Na₂S₂O₃ solution using 1ml starch as indicator.
6. At the end point the blue colour solution changes to colourless.
7. Note down the concordant volume of hypo solutions as V ml.

TABULATION:

CALCULATION:

RESULT:

Amount of total residual chlorine in a given sample of water = ppm

PRECAUTIONS:

1. During weighing the sample bottle should be kept stoppered, otherwise chlorine evolved, due to decomposition of the sample may corrode the material of the balance.
2. The solution being unstable should be titrated immediately after its preparation.
3. The solution should be well shaken before each aliquot is withdrawn for titration.
4. Chlorine vapours being harmful, the solution should not be sucked into the pipette with the mouth.

EXPERIMENT 13

OBJECTIVE:

To determine chloride ion in a given water sample by Argentometric method (Mohr's Method)

APPARATUS REQUIRED:

1. Burette
2. Pipette
3. Conical flask
4. Measuring flask

CHEMICALS REQUIRED:

1. Standard silver nitrate solution (N/50)
2. Potassium chromate indicator solution (K_2CrO_4)

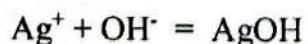
THEORY:

Chlorides are present in water usually as $NaCl$, $MgCl_2$ and $CaCl_2$. Although chlorides are not harmful as such, their concentration over 250ppm imparts a peculiar taste to the water thus rendering the water unacceptable for drinking purpose.

Further existence of unusually high concentrations of chloride in a water sample indicates pollution from domestic sewage or from industrial waste waters. Salts like $MgCl_2$ may undergo hydrolysis under the high pressure and temperature prevailing in the boiler, generating hydrochloric acid which causes corrosion in boiler parts chlorides in the form of $MgCl_2$ and $CaCl_2$ cause Permanent hardness & area source of trouble not only at our house hold but also in industries.

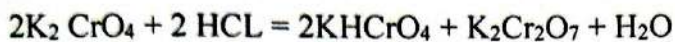
By Argentometric method, chlorides in a water sample which is neutral or slightly acidic can be determined by it against standard silver nitrate solution using potassium chromate.

The p^H should be in between 7-8



And at lower p^H ,

K_2CrO_4 indicator is converted to $K_2Cr_2O_7$.



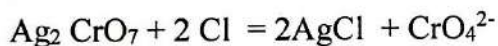
(Pot. Chromate)

(Pot. dichromate)

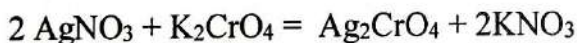
$KHCrO_4$ is weak in nature. Concentration of CrO_4^- decreases & therefore higher concentration of Ag^+ is needed for the soluble Ag reacts with Cl^- and CrO_4^{2-} when $AgNO_3$ solution is released from the burette to the sample solution which as Cl^- .



Red colour formed because of formation of silver chromate disappears as the solution contains high concentration of Cl^- .



When the concentration of chloride ions has decreased the red colour starts disappearing slowly and slowly on shaking and a stage is reached when all the chloride ions have formed $AgCl$. One extra drop of $AgNO_3$ at this point reacts with potassium chromate and reddish coloured silver chromate is formed.



PROCEDURE:

(i) Titration with distilled water:

1. Transfer 50ml of distilled water in a conical flask and add 3-4 drops of potassium chromate indicator
2. Slowly add standard solution of $AgNO_3$ solution from the burette and the volume of titrant is noted down as an end point when yellow colour starts changing to red colour.
3. The titration is repeated until concordant volume V_1 is obtained.

4. The blank correction for the indication should be deducted from the volume of the titrants obtained after titrating the sample solution in steps 2.

(ii) Titration with the Sample solution:

1. Transfer 50ml of given water sample in a conical flask. Add 3-4 drops of freshly prepared potassium chromate solution.
2. Titrate it against AgNO_3 solution and shake the solution. At the end point light yellow colour starts changing to red colour and red colour persists.
3. Repeat the titration until the concordant volume is obtained.
4. Say the volume of $\text{N}/50 \text{ AgNO}_3$ consumed is V_2 ml.

TABULATION:

CALCULATIONS:

CONCLUSION:

From the above experiment the amount of chloride ion in a given water sample is ppm.

PRECAUTIONS:

1. The whole apparatus should be washed with distilled water before the start of the experiment.
2. The reaction mixture should be briefly shaken during the titration.
3. The end point of the reaction should be carefully observed.
4. the volume of the indicator should be same in all the titration.
5. The p^H of the sample solution should be adjusted to 7-8 range by adding acidic or basic solution to the sample solution.