Pharmacology & Toxicology Lab

BML-305

Determination Of Dissolved Oxygen In Effluent Sample By Winkler's Method

Object: Determination of dissolved oxygen in effluent sample by Winkler's method

Theory: Dissolved oxygen (DO) is an index of physical and biological processes going on in water. Non polluted surface water are normally saturated with dissolved oxygen. It comes in H_2O from 2 main sources

1. Diffusion from air

2. Photosynthetic activity with in water

Diffusion of O_2 from air to water is a physical phenomenon and is influenced by factors which effect the oxygen solubility like temperature, water movements and salinity etc.

Photosynthetic activity is a biological phenomenon carried out by autotrophs (mainly phytoplankton in water) and depends upon autotrophs population, light conditions and available gasses etc.

Oxygen is considered to be a limiting factor especially in lakes and in waters with a heavy load of organic materials. Organisms have specific oxygen requirements. Low dissolved oxygen may prove lethal for many organisms. Dissolved oxygen in water can be determined by 2 methods

(i) Oxygen meter method

(2) Iodometric method (Winkler's method).

Principle: Oxygen combines with $Mn(OH)_2$ forming higher hydroxides which on acidification in the presence of iodide, liberate iodine in an amount equivalent to the original dissolved oxygen content of the sample. The iodine is then determined by titration with sodium thiosulphate solution ($Na_2S_2O_3$)

Materials and Reagents:

1. BOD bottles (100-300 ml)

2. Manganous sulphate solution: Dissolve 100 g of manganous sulphate in 200 ml of previously boiled distilled water and filter the solution.

3. Alkaline potassium iodide solution: Dissolve separately 350 g of KOH and 75 g of KI in distilled water, mix the two and make the volume 500 ml with distilled water Dissolve separately 5 g of sodium azide in 20 ml of distilled water. Mix alkaline iodide and sodium azide solutions. Sodium azide avoids the interference due to organic matter and chlorides present in sample.

4. Sodium thiosulphate solution (0.025 N): Take 6.205 g of sodium thio sulphate and dissolve it in previously boiled distilled water and make up the volume to 1 litre. Add a pallet of NaOH as a preservative. Keep the solution in coloured bottle.

5. Starch indicator: Dissolve 1 g of starch in 100 ml of warm distilled water and add a few drops of Toluene or formal dehyde as preservative.

6. Concentrated H_2SO_4 : SP gravity 1.84 and 18 M.

Method:

- Take a glass stoppered BOD bottle of known volume (100-300 ml) and fill it with sample avoiding any bubbling. No air should be trapped in bottle after the stopper is placed.
- Open the bottle and add in it 1 ml of each manganous sulphate and alkaline KI solutions using separate pipettes. If the volume of sample is over 200 ml add 2 ml of each reagent instead of 1 ml.
- A precipitate will appear. Place the stopper and shake the bottle thoroughly. Sample at this stage can be stored for a few days, if required.
- Add 2 ml of H_2SO_4 and shake thoughly to dissolve the precipitate.
- Transfer gently (avoid bubbling) whole content in a conical flask add few drops of starch indicator.
- Titrate against sodium thiosulphate solution and note the endpoint reading when initial blue colour turns to colourless.

Calculation: $DO\ (mg/L) = \frac{V1\ N \times 8 \times 1000}{V2 - V3}$

Where, DO = Dissolved oxygen

V1 = Volume of titrant ($Na_2S_2O_3$) (ml)

N = Normality of titrant (0.025 N)

V2 = Volume of sample after placing the stopper

V3=Volume of manganous sulphate + alkaline KI solutions added (ml.)

Equivalent weight of oxygen * To obtain the value of DO in ml/L divide the DO in mg/l by 1.43

Result: The dissolved oxygen in sample is .....mg/L and .....ml/L

DETERMINATION OF ASPIRIN IN TABLETS USING BACK TITRATION

Aim: To calculate and compare the active pharmaceutical ingredient (API) in different commercially available aspirin tablets of the same batch using titration technique.

STANDARD(S) & INDICATOR(S):

- Identify questions and make predictions that can be addressed by conducting investigations.
- Collect, organize, and interpret the data that result from experiments.
- Recognize evidence of a chemical change.

OBJECTIVE(S): Students will be able to:

1. Students will be able to calculate the amount of the active pharmaceutical ingredient(API) in aspirin tablets by performing acid-base titration
2. Students will be able to compare the amounts of aspirin in different tablets of the same batch.
3. Students will be able to evaluate the mixing efficiency of components in a tablet by analyzing the results

Materials Needed

Aspirin tablets, 0.1 M Hydrochloric acid, 0.1 M Sodium hydroxide solution, Ethanol, Phenolphthalein indicator, Burettes, Mortar & Pestle, Balance, Water bath.

Theory:

The key concepts from the research work are the following:
1. Importance of particle size in industrial applications
2. Importance of proper mixing of substances in industry.

Hypothesis: All tablets contain the same amount of API mentioned in the label described by the manufacturer.
Many reactions are slow or present unfavorable equilibria for direct titration. Aspirin is a weak acid that also undergoes slow hydrolysis; i.e., each aspirin molecule reacts with two hydroxide ions. To overcome this problem, a known excess amount of base is added to the sample solution and an HCl titration is carried out to determine the amount of unreacted base. This is subtracted from the initial amount of base to find the amount of base that actually reacted with the aspirin and hence the quantity of aspirin in the analyte.

Safety Issues and Chemical Hazard Information

Physical Hazards Health Hazards

Aspirin none toxic, irritant, sensitizer
Ethanol flammable irritant
Hydrochloric acid water-reactive, corrosive toxic
Phenolphthalein none irritant
Sodium hydroxide water-reactive, corrosive toxic, irritant
Concentrated hydrochloric acid is highly corrosive. Be careful. Ethanol is flammable. Keep it away from heat.

Procedure:

Sample preparation

1. Accurately record the weight of a group of three aspirin tablets so that you can determine an average tablet weight. Use a mortar and pestle to crush enough tablets to produce approximately 1 g tablet powder.
2. Weigh approximately 300 mg aspirin powder, into labeled 250 mL Erlenmeyer flasks.
3. To each flask, add 20 mL of ethanol (measure by graduated cylinder) and three drops of phenolphthalein indicator. Swirl gently to dissolve. (Aspirin is not very soluble in water — the ethanol helps the aspirin dissolve. Note that an aspirin tablet contains other compounds in addition to aspirin. Some of these are not very soluble. Your solution will be cloudy due to insoluble components of the tablet.)

Aspirin Titration with Base

4. Titrate the first aspirin sample with NaOH to the first permanent cloudy pink color.
5. The aspirin/NaOH acid-base reaction consumes one mole of hydroxide per mole of aspirin. The slow aspirin/NaOH hydrolysis reaction also consumes one mole of hydroxide per mole of aspirin, and so for a complete titration we will need to use a total of twice the amount of NaOH that you have already used, plus we will add some excess NaOH to ensure we really have reacted with all of the aspirin in the sample.
(For example: if you used 26 mL of base in the previous step, the
volume of base you would add now would be 26 + 10 = 36 mL. Thus, you would have added a total of 26 + 26 + 10 = 62 mL of base.)

Heating for Completion of Reaction

6. Heat gently the flask contents in a water bath. Avoid boiling, because the sample may decompose. While heating, swirl the flasks occasionally. After 15 minutes, remove samples from the water bath and cool for 5 minutes.
7. If the solution is colorless, add a few more drops of phenolphthalein. If it remains colorless, add 10 mL more of the base and reheat. (**Don't forget** to add this additional volume of base to the previously recorded total volume.)
8. The only base remaining in each flask will be excess base that has not reacted with the aspirin. Using your burette with your ~0.1 M HCl solution, titrate the excess base in each flask with HCl until the pink color just disappears. The endpoint is best described as —cloudy white‖.
9. Record all the volumes of bases and acid in the data table.

Data & Calculations

Average mass of an aspirin tablet =
Mass of aspirin tablet powder used in the experiment =
Volume of the base (NaOH) used in the first titration (V1) =
Volume of the extra base (NaOH) added in the flask (V2) =
Total volume of the base (NaOH) used in the reaction (V3) =
Volume of the acid (HCl) reacted with the mixture in the second titration (V4) =
Actual volume of the base (NaOH) reacted (V5) = V3 – V4
Number of moles of NaOH reacted = Volume of the base in liters x Molarity of the base
Number of moles of aspirin reacted = 1 mole aspirin x moles of NaOH reacted
2 moles of NaOH
Mass of aspirin reacted = 180.2 g x moles of aspirin
1 mole aspirin

Pre-Lab questions:

Define the following terms:
Acids, bases, titration, endpoint, indicator, molarity

Post-Lab questions (Show all calculations where ever necessary)

1. Write a balanced chemical equation for the reaction between hydrochloric acid and sodium hydroxide
2. Write the skeletal and balanced chemical equation for the reaction between aspirin and sodium hydroxide.
3. What is the actual volume of sodium hydroxide used to neutralize aspirin in the experiment?
4. How many grams of sodium hydroxide reacted in the experiment?
5. How many grams of aspirin (API) present in each tablet?
6. What is the percentage of aspirin in each tablet?
7. Compare your results with different groups. Explain your finding.
8. Based on your findings from different groups, what is your conclusion about mixing efficiency of different components in a tablet?

Assessment

Pre lab questions – 3 points
Calculations & Data Analysis – 5 points
Post lab questions based on the
experimental reactions and calculations – 5 points
Lab report containing discussion & Conclusion - 10 points
Presentation/ Impression – 2 points

ESTIMATION OF TOTAL, PERMANENT AND TEMPORARY HARDNESS OF WATER (EDTA METHOD)

Expt. No.: Date:

Aim: To estimate the amount of total, permanent and temporary hardness in the collected sample of water. A standard solution of ..... EDTA is provided.

Principle: Hardness in water is due to the presence of dissolved salts of calcium and magnesium. It is unfit for drinking, bathing, washing and it also forms scales in boilers. Hence it is necessary to estimate the amount of hardness producing substances present in the water sample. Once it is estimated, the amount of chemicals required for the treatment of water can be calculated.

The estimation of hardness is based on complexometric titration. Hardness of water is determined by titrating with a standard solution of ethylene diamine tetra acetic acid (EDTA) which is a complexing agent. Since EDTA is insoluble in water, the disodium salt of EDTA is taken for this experiment. EDTA can form four or six coordination bonds with a metal ion.

1. **Total hardness**: Total hardness is due to the presence of bicarbonates, chlorides and sulphates of calcium and magnesium ions. The total hardness of water is estimated by titrating the water sample against EDTA using Eriochrome Black-T (EBT) indicator. Initially EBT forms a weak EBTCa2+/Mg2+ wine red coloured complex with Ca2+/Mg2+ ions present in the hard water.

   On addition of EDTA solution, Ca2+/Mg2+ ions preferably forms a stable EDTACa2+/Mg2+ complex with EDTA leaving the free EBT indicator in solution which is steel blue in colour in the presence of ammonia buffer (mixture of ammonium chloride and ammonium hydroxide, pH 10).

2. **Temporary hardness:** Temporary hardness is due to the presence of bicarbonates of calcium and magnesium ions. It can be easily removed by boiling. When water is boiled, temporary hardness producing substances (bicarbonates) are precipitated as insoluble carbonates or hydroxides. This precipitate can be removed by filtration. (The filtrate is used in the next step)

3. **Permanent hardness** Permanent hardness is due to the presence of chlorides and sulphates of calcium and magnesium ions. This type of hardness cannot be removed by boiling. The filtrate obtained from the above step contains permanent hardness producing substances and is estimated against EDTA using EBT indicator.

   Procedure: The burette is filled with standard EDTA solution to the zero level, following usual precautions.

   - **Estimation of Total Hardness** 20 ml of the given water sample is pipetted out into a clean conical flask. 5 ml ammonia buffer and 2 drops of EBT indicator are added and titrated against EDTA from the burette. The end point is the change of colour from wine red to steel blue. The titration is repeated to get concordant titre value. Eriochrome Black-T + Ca 2+ /Mg 2+ Eriochrome Black-T-Ca 2+ /Mg 2+ Eriochrome Black-T-Ca 2+ /Mg 2+ + EDTA EDTA-Ca 2+ /Mg 2+ + Eriochrome Black-T (Wine red) (Wine red) (Steel blue) Eriochrome Black-T + Ca 2+ /Mg 2+ Eriochrome Black-T-Ca 2+

/Mg 2+ Eriochrome Black-T-Ca 2+ /Mg 2+ + EDTA EDTA-Ca 2+ /Mg 2+ + Eriochrome Black-T (Wine red) (Wine red) (Steel blue)

- Estimation of Permanent Hardness 100 ml of the given sample of water is pipetted out into a clean beaker and boiled for 20 minutes. It is then filtered to remove the precipitate formed due to the decomposition of temporary hardness producing salts. The filtrate is made up to 100 ml in standard measuring flask (SMF) using distilled water. 20 ml of the made up solution is pipetted out into a conical flask, 5 ml ammonia buffer and 2 drops of EBT indicator are added and titrated against the EDTA. The end point is the change of colour from wine red to steel blue. The titration is repeated to get concordant titre value.

4. **Temporary Hardness** The temporary hardness is calculated from the total and permanent hardness. Temporary Hardness = Total Hardness – Permanent Hardness

   Result: The collected water sample contains Total hardness = ppm Permanent hardness = ppm Temporary hardness = ppm

   Titration-1 Estimation of Total Hardness Standard EDTA vs Water sample Volume of hard water sample (ml) Burette Reading Volume of EDTA solution (ml) Indicator Initial Final

   Titration-2 Estimation of Permanent Hardness Standard
   EDTA X Boiled water sample Volume of boiled water sample (ml) Burette Reading Volume of EDTA solution (ml) Indicator Initial Final

   Calculation:

   1 ml of 0.01 M EDTA ≡ 1 mg of $CaCO_3$ V1 ml of EDTA ≡ V1 mg of $CaCO_3$ Calculation of total hardness

   Volume of EDTA solution consumed = ... ml

   Volume of hard water taken = ... ml

   Total hardness = Volume of EDTA solution consumed X1000 Volume of the hard water taken ppm = ... ppm

   Calculation of permanent hardness Volume of EDTA solution consumed = ... ml Volume of boiled water taken = ... ml

   Permanent Hardness = Volume of EDTA solution consumed X1000 Volume of the boiled water taken ppm = ... ppm

CALCULATION ON LD50 VALUE OF AN INSECTICISED FROM THE DATA PROVIDED

Introduction

Pesticide applicators should understand the hazards and risks associated with the pesticides they use. Pesticides vary greatly in **toxicity**. Toxicity depends on the chemical and physical properties of a substance, and may be defined as the quality of being poisonous or harmful to animals or plants. Pesticides have many different modes of action, but in general cause biochemical changes which interfere with normal cell functions.

The toxicity of any compound is related to the dose. A highly toxic substance causes severe symptoms of poisoning with small doses. A substance with a low toxicity generally requires large doses to produce mild symptoms. Even common substances like coffee or salt become poisons if large amounts are consumed.

Toxicity can be either acute or chronic.

- **Acute toxicity** is the ability of a substance to cause harmful effects which develop rapidly following exposure, i.e. a few hours or a day.
- **Chronic toxicity** is the ability of a substance to cause adverse health effects resulting from long-term exposure to a substance.

There is a great range in the toxicity of pesticides to humans. The relative **hazard** of a pesticide is dependent upon the toxicity of the pesticide, the dose and the length of time exposed. The hazard in using a pesticide is related to the likelihood of exposure to harmful amounts of the pesticide. The toxicity of a pesticide can't be changed but the risk of exposure can be reduced with the use of proper personal protective equipment (PPE), proper handling and application procedures.

Pesticide Toxicity

Some pesticides are dangerous after one large dose (acute toxicity). Others can be dangerous after small, repeated doses (chronic toxicity).

Measuring Acute Toxicity (LD50And LC50 Values)

The smaller the LD50, the more toxic the pesticide.

Example: a pesticide with an LD50 of 5 mg/kg is 100 times more toxic than a pesticide with an LD50 of 500 mg/kg

Acute toxicity of a pesticide refers to the effects from a single dose or repeated exposure over a short time (e.g. one day), such as an accident during mixing or applying pesticides. Acute toxicity is measured by LD50 and LC50 values.

The LD50 value is the amount of pesticide (lethal dose) which kills 50% of the test animals. These treatments are through the skin (dermal) or through the mouth (oral).

values are given in milligrams per kilogram of body weight of the animal (mg/kg body wt.). A pesticide with a lower LD50 is more toxic than a pesticide with a higher number because it takes less of the pesticide to kill half of the test animals.

The LC50 value is a measure of the toxicity of a pesticide when test animals breathe air mixed with pesticide dust, vapours or spray mist. The LC50 is the concentration of pesticide which is lethal to 50% of a population of test animals and is usually determined for a specific exposure period (e.g. inhalation for 4 hours). The length of exposure is important because shorter exposure periods generally require higher pesticide concentrations to produce toxic effects. LC50 values for pesticides in air are expressed as the ratio of pesticide to air, in parts per million (ppm) or parts per billion (ppb). LC50 values are also determined for fish and aquatic organisms based on the concentration of pesticide in water.

Important characteristics to note about LD50 and LC50 values:

- they are based on a single dose (LD50) or short exposure (LC50);
- they do not indicate cumulative effects of small doses;
- they are an indicator of the amount of chemical required to kill or severely injure animals, and do not indicate the amount of chemical causing less severe toxic effects.

Relation of oral LD50 to approximate lethal dose in adult humans.

Oral LD50	**Approximate lethal dose to average size adult* (70 kg or 155 lb.)**
less than 50 mg/kg	0.3 to 3 mL (a few drops to half a teaspoon)
50 to 500 mg/kg	3 mL to 30 mL (half a teaspoon to two tablespoons)
500 to 5,000 mg/kg	30 mL to 300 mL (1 to 10 fluid ounces)
5,000 to 15,000 mg/kg	300 mL to 900 mL (10 to 30 fluid ounces)

Note that a child who is one-fifth the weight of an adult would require only one-fifth the amount of pesticide to suffer the same toxic effects as the adult.

Pesticides are grouped according to their LD**50** values. The groups are Very Toxic, Moderately Toxic and Slightly Toxic.

WorkSafeBC defines these groups as shown in the following table. Note that these definitions are not the same as the criteria used by the Pest Management Regulatory Agency for use of warning symbols on product labels.

Relative toxicity of pesticides to humans based on acute oral and dermal LD50's.		
Acute Toxicity	**Oral LD50**	**Dermal LD50**
Very	less than 50 mg/kg	less than 200 mg/kg
Moderately	50 to 500 mg/kg	200 to 1,000 mg/kg
Slightly	over 500 mg/kg	over 1,000 mg/kg

Here are examples of LD50 values for four pesticides.

Pesticide Active Ingredient (a.i.)	LD50 (mg/kg)	
Oral	Dermal	
aldicarb	0.8	3
diazinon	300	2,150
malathion	1,000	4,100
atrazine	1,780	7,500

Here are examples of LD50 values for three common household compounds. They have a low acute toxicity but could cause toxic reactions if consumed in sufficient quantities.

Compound	Oral LD50 (mg/kg)
acetylsalicylic acid (Aspirin)	1,000
sodium chloride (table salt)	3,320
ethylene glycol (antifreeze)	460

Labels and Toxicity Symbols				
[No symbol]				
Poison Hazard Symbol	Danger Poison	Warning Poison	Caution Poison	Very low toxicity
Acute oral LD50	less than 500 mg/kg	500 - 1000 mg/kg	1000 - 2000 mg/kg	greater than 2500 mg/kg
Acute dermal LD50	less than 500 mg/kg	500 - 1000 mg/kg	1000 - 2000 mg/kg	greater than 2500 mg/kg

Chronic Toxicity

Chronic toxicity refers to the effects of long-term or repeated lower level exposures to a toxic substance, such as when a pesticide applicator is frequently wetted with spray during unsafe spray practices. The effects of chronic exposure do not appear immediately after first exposure and may take years to produce symptoms. Pesticides which have a tendency to accumulate, or which break down slowly in body tissues, usually represent the greatest chronic exposure hazard. Someone who is frequently exposed to low doses of such pesticides may develop symptoms of poisoning long after the first exposure. Chronic exposure may include chronic oral, chronic dermal or chronic inhalation poisoning.

Exposure

Human Pesticide Exposure

There are three ways in which pesticides can enter the human body:

1. through the skin or eyes (dermal),
2. through the mouth (oral) and
3. through the lungs (respiratory or inhalation).

Dermal Exposure

In typical work situations, skin absorption is the most common route of pesticide poisoning. Absorption will continue as long as the pesticide remains in contact with the skin. The rate of absorption is different for each part of the body (see diagram). The head (especially the scalp and ear canal) and the genital areas are particularly vulnerable. Absorption may occur as a result of a splash, spill or drift when mixing, loading or applying a pesticide. Applicators may also be exposed to residues on application equipment, protective clothing or treated surfaces after pesticide application. Following exposure, residues can also be transferred from one part of the body to another. A cut or skin abrasion can greatly increase pesticide absorption.

The dermal toxicity of a pesticide depends on the pesticide formulation, the area of the body contaminated and the duration of the exposure. In general, liquids are more easily absorbed through the skin than wettable powders or granules. The hazard from skin absorption increases when workers are mixing pesticides because they are handling concentrated pesticides that contain a high percentage of active ingredient.

Protect yourself from dermal exposure. Follow these guidelines:

1. Wear protective clothing and equipment when using pesticides or repairing contaminated equipment.
2. Spray during periods when there is little or no wind.
3. Do not re-enter a sprayed field without protective clothing until the re-entry time has elapsed.
4. If your clothes become contaminated, change immediately. Wash affected areas of the skin.
5. Change clothes as part of the clean-up after pesticide use at the end of the day.
6. Wash and shower after using pesticides.
7. Wear clean clothes at the start of each day during pesticide application.

Eye Exposure

The tissues of the eyes are particularly absorbent. Enough pesticide can be absorbed through the eyes to result in serious or fatal poisoning. In addition, some pesticides may cause chemical injury to the eye itself. Eye protection is needed when measuring or mixing concentrated or highly toxic pesticides. Protective face shields or goggles should be worn whenever there is a chance that pesticide sprays or dusts may come in contact with the eyes.

Protect yourself from eye exposure. Follow these guidelines:

1. Always wear eye protection when you measure or mix pesticides.
2. Always wear eye protection when pesticide sprays or dusts may contact your eyes.
3. Do Not wipe your eyes with contaminated gloves or hands.
4. Be prepared to respond to accidental eye exposure quickly (see Pesticide Poisoning).

Oral Exposure

Pesticides taken through the mouth result in the most severe poisoning, compared to other types of exposure. Pesticides can be ingested by accident, through carelessness, or intentionally. The most frequent cases of accidental oral exposure are those in which pesticides have been stored in an unlabelled bottle or food container. There are many cases where people, especially children, have been poisoned by drinking pesticides from a soft drink bottle. People have also been poisoned by drinking water stored in contaminated containers. Workers handling pesticides or application equipment can also consume excessive levels of pesticides if they do not wash their hands before eating or smoking.

Protect yourself from oral exposure. Follow these guidelines:

1. Always store pesticides in their original labeled containers.
2. Never put pesticides in an unlabelled bottle or food container.
3. Never use your mouth to clear a spray hose or nozzle, or to begin siphoning a pesticide.
4. Always wash after handling pesticides and before eating, drinking, smoking, or using the toilet.
5. Never leave pesticides unattended.
6. Avoid splashes or dusts when mixing pesticides.
7. Label your pesticide measuring containers.

Respiratory Exposure

Certain pesticides may be inhaled in sufficient amounts to cause serious damage to nose, throat and lung tissues, or to be absorbed through the lungs into the bloodstream. Vapours and very small particles pose the most serious risks. The hazard of poisoning from respiratory exposure is great because of the rapid and complete absorption of pesticides through lung tissues.

Lungs may be exposed to pesticides by inhalation of powders, airborne droplets or vapours. Working with wettable powders can be hazardous because the powder may be inhaled during mixing operations and usually contains concentrated pesticide active ingredient. The hazard from inhalation of pesticide spray droplets is fairly low when dilute sprays are being applied with conventional low pressure application equipment. This is because most droplets are too large to remain airborne and be inhaled. However, when high pressures are used or ultra-low volume (ULV) or fogging equipment are used, the potential for respiratory exposure is increased. The droplets produced during these operations are in the mist or fog size-range and can be carried on air currents for a considerable distance.

Many pesticides give off a vapour when exposed to air. As temperatures increase, vapour levels of many pesticides increase. Fumigants are used because their toxic vapours are desirable for pest control. They also have the highest hazard with respect to worker exposure to vapours. Some non-fumigant pesticides are toxic to pests as liquid or solid formulations, but also give off vapours which could be toxic to applicators or bystanders. The hazard is greatest in enclosed spaces where there is little air movement. For example, high vapour levels could result from a spill in an unventilated storage area or application in a confined space such as a greenhouse. Air currents due to wind or ventilation can substantially reduce vapour levels.

Many pesticides that produce vapor's provide a warning of their presence by their smell or by causing irritation of the eyes, nose and throat. However, some pesticide vapours have little smell and provide little warning of their presence.

Pesticides with high vapour hazards will have label directions to use respiratory protection equipment. Protect yourself from respiratory exposure. Follow these guidelines:

1. Wear an appropriate and properly fitting respirator:
1. If it is required on the label;

2. If pesticides are used or mixed in poorly ventilated areas.

3. If there is a possibility of inhaling spray droplets, vapour, or powder.
2. Do not re-enter a treated area too soon. Follow the re-entry guidelines on the label.

3. Ventilate greenhouses or enclosed structures after pesticide application, before re-entry.

4. Do not apply pesticides when air temperatures are above 30°C.

Toxicity of pesticides can vary depending on the type of exposure; dermal, oral or respiratory (inhalation), but it is important to remember that, in each case, the danger usually increases as concentration and duration of exposure increases. The longer a pesticide remains on the skin or in eyes, or the longer it is inhaled, the greater the damage that is likely to result.

Interpretation of Symbol Combinations Poisonous Hazard:	DANGER POISON	WARNING POISON	CAUTION POISON
Acute oral LD50	< 500 mg/kg	500 - 1,000 mg/kg	1,000 - 2,000 mg/kg
Acute dermal LD50	< 500 mg/kg	500 - 1,000 mg/kg	1,000 - 2,000 mg/kg
Respirator	yes	advisable in confined spaces	advisable in confined spaces
Eye Protection	yes	yes	advisable
Eye Effects	corrosive or irreversible	severe but reversible	irritation
Petroleum Distillates	10% or more	1% to 10%	-

Flammability Hazard:	DANGER FLAMMABLE	WARNING FLAMMABLE	CAUTION FLAMMABLE
Liquid Products - Flash Point	< -6°C (20°F)	-6° to 10°C (20° to 50°F)	10° to 27°C (50°to 80°F)
Pressurized Products - Flame Projection	45 cm (18")	15-45 cm (6 to 18")	< 15 cm (6")

Corrosive Hazard:	DANGER CORROSIVE	WARNING CORROSIVE	CAUTION CORROSIVE
acid or alkali materials	10% or more	5 to 10%	1 to 5%
organic acids	20% or more	5 to 20%	1 to 5%
available chlorine	-	10% or more as liquid	over 1% as solids
pH	-	0.5 or 13.5	0.5 to 2.5 or 11.5 to 13.5
Available chlorine > 4% and < 10% as liquid		CAUTION IRRITANT	
Available chlorine > 1% and < 4% as liquid		CAUTION	

INTRODUCTION

The acid value (AV) is a common parameter in the specification of fats and oils. It is defined as the weight of KOH in mg needed to neutralize the organic acids present in 1g of fat and it is a measure of the free fatty acids (FFA) in a sample of oil or fat indicates hydrolysis of triglycerides. Such reaction occurs by the action of lipase enzyme and it is and indicator of inadequate processing and storage conditions (i.e. high temperature, and relative humidity, tissue damage). Besides of free fatty acids, hydrolysis of triglycerides produces glycerol.

$$RCOOH + KOH \rightarrow ROO^{-}\ K^{+} + H_2O$$

Free fatty acids are a source of flavors and aromas. On one side, short chain of free fatty acids tend to be water soluble and volatile with characteristic smell. On the other hand, we have long chain saturated and unsaturated fatty acid. The later are more prone to oxidation in their free form and their breakdown products (aldehydes, ketones, alcohols, and organic acids) provide characteristic flavors and aromas. In most cases, these flavor and aromas are considered a defect in oils, fats, and foods that contain them. However, there are instances where hydrolysis of triglycerides and oxidation of free fatty acid are key in the development of desirable flavor and aroma in foods. This is the case of aged cheeses and some processed meats.

There are standard methods for determining the acid number, such as ASTM D 974 and DIN 51558 (for mineral oils, biodiesel), or specifically for Biodiesel using the European Standard EN 14104 and ASTM D664 are both widely utilised worldwide. Acid number (mg KOH/g oil) for biodiesel should to be lower than 0.50 mg KOH/g in both EN 14214 and ASTM D6751 standard fuels. This is since the FFA produced may corrode automotive parts and these limits protect vehicle engines and fuel tanks.

As oil-fats rancidify, triglycerides are converted into fatty acids and glycerol, causing an increase in acid number. A similar observation is observed with Biodiesel aging through analogous oxidation processes and when subjected to prolonged high temperatures (ester thermolysis) or through exposure to acids or bases (acid/base ester hydrolysis).

THEORY

Different fat samples may contain varying amount of fatty acids. In addition, the fats often become rancid during storage and this rancidity is caused by chemical and enzymatic hydrolysis of fats into free acids and glycerol. The amount of free fatty acids can be determined volumetrically by titrating the sample with potassium hydroxide. The acidity of fats and oils is expressed as its acid value or number which is defined as mg KOH required to neutralize the free fatty acids present in 1g of fat or oil. The amount of free acids present or acid value of fat is a useful parameter which gives an indication about the age and extent of its deterioration.

TITLE

Determination of acid value of fats and oils

APPARATUS

Beakers, Conical flask, Pipette, Burette, Retort stand

MATERIALS

0.1N Oxalic acid, Potassium hydroxide (KOH) solution, Phenolphthalein indicator, Fat solvent, Fat sample

PROCEDURE

(A)Standardize normality of potassium hydroxide solution, KOH

(1) 10ml of 0.1N oxalic acid is poured into conical flask. 2-3 drops of phenolphthalein indicator is added to the solution.

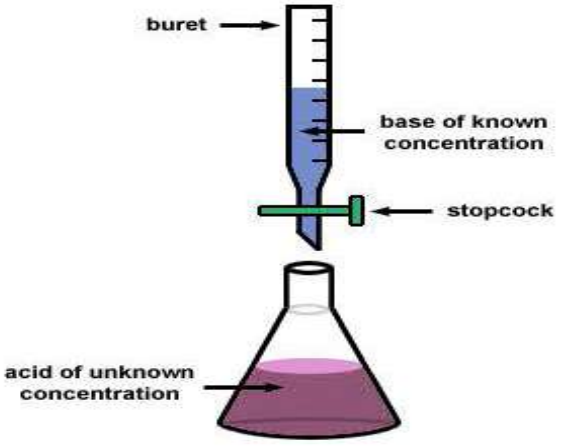
(2) The solution is titrated with KOH solution till a permanent pink colour appears. The volume of KOH solution used is recorded . Using the volume of KOH solution is used, the concentration of KOH is calculated by using the formula $S_1V_1=S_2V_2$.

(B)Determination of acid value of fats and oils

(1) 5g of fat sample is weighed and placed in a conical flask. Then, 25ml of fat solvent is added to the conical flask and shake it well. Few drops of phenolphthalein is added to the solution also.

(2) The above solution is titrated with 0.1N KOH until a faint pink colour persists for 20-30 seconds. The volume used is recorded.

(3) Step 1 and 2 above is repeated with a blank sample which does not contain any fat sample.



RESULTS AND CALCULATIONS

(A)Standardize normality of potassium hydroxide solution, KOH

Table of volume of KOH used in titration

No of Observation	Initial Reading (ml)	Final Reading (ml)	Average Reading (ml)
1	0.00	17.00	17.00
2	0.00	16.90	
3	0.00	17.10	

$V_1S_1=V_2S_2$

where V_1= Volume of oxalic acid used

S_1= Normality of oxalic acid,

V_2= Volume of KOH used

S_2= Normality of KOH

$$S_2 = \frac{V_1 S_1}{V_2}$$

$$= \frac{(10.00ml)(0.1N)}{(17.00ml)}$$

$$= 0.05882N$$

Therefore, normality of KOH is 0.05882N.

(B)Determination of acid value of fats and oils

Table of volume of KOH used in titration(with fat sample)

No of Observation	Fat sample Used (g)	Volume of fat solvent(ml)	Initial Reading (ml)	Final Reading (ml)	Average Reading (ml)
1	4.995	25.00	0.00	0.50	0.50
2	4.995	25.00	0.00	0.50	

Table of volume of KOH used in titration(without fat sample)

No of Observation	Volume of fat solvent used (ml)	Initial Reading (ml)	Final Reading (ml)	Average Reading (ml)
1	25.00	0.00	0.10	0.10
2	25.00	0.00	0.10	

0.1N KOH solution used for blank sample (Blank) = 0.10ml

0.1N KOH solution used for sample (With fat sample) = 0.50ml

Titre value for sample = 0.50ml – 0.10ml

= 0.40ml

Therefore, titre value of sample is 0.40ml.

$$\text{Acid value (mg KOH/g fat)} = \frac{\text{Titre value x Normality of KOH x 56.1}}{\text{Weight of sample (g)}}$$

1ml of 0.1N KOH contains 56.1mg of KOH. Hence a factor of 56.1 is incorporated in the numerator in the above equation to obtain weight of KOH from the volume of 0.1N KOH solution used during this titration.

$$\text{Hence, 0.05882 N KOH contains } \frac{56.1\text{mg x } 0.05882\text{N}}{0.1\text{N}} = 32.998\text{mg}$$

$$\text{Acid value (mg KOH/ g fat)} = \frac{(0.40\text{ml}) \times (0.05882\text{N}) \times (32.998\text{mg})}{(4.995\text{g})}$$

$$= \underline{0.1554 \text{ mg KOH/ g fat}}$$

Therefore, Acid value of the fat sample after calculated is 0.155 mg KOH/ g fat.

DISCUSSION

Acid value defined as the amount of potassium hydroxide in milligram required to neutralize the free fatty acids (FFAs) in one gram of fats and oils. . The acid number is a measure of the amount of carboxylic acid groups in a chemical compound, such as a fatty acid, or in a mixture of compounds. In a typical procedure, a known amount of sample dissolved in organic solvent (often isopropanol), istitrated with a solution of potassium hydroxide with known concentration and with phenolphthalein as a colour indicator. From the results, we can see that 0.1554mg of KOH is needed to neutralize 1 gram of fat and oils.

The majority of national and international standards for AV determination in fat and oils are based on the acid-base titration techniques in non-aqueous solvents. However, these techniques have a number of drawbacks:

- Currently used non-aqueous solvents are toxic, such as ethanol or isopropanol heated up to 60°C or higher or diethyl ether-ethanol solvent.
- Incomplete solubility of test oil portion in alcohol (even under heating) caused by the formation of a dispersed system.
- Conditions for accurate acid-base titration in hot amphoteric solvents might deteriorate due to the increase of the solvolysis constant for anion titrable weak acids with an increase in temperature. This conclusion follows from the fact that the solvent auto-protolysis constant increases, and the acid dissociation constant decreases with increased temperature.
- Need to previously neutralize the solvents.

CONCLUSION

The acid value of the provided sample is 0.1554mg KOH / g fat.

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