

Laboratory Manual

B.Sc.(Honours Chemistry) 5TH Semester



RPS DEGREE COLLEGE

BALANA (MAHENDERGARH)123029

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ORGANIC CHEMISTRY

1 Experiment: - 1

(A). To separate a mixture of O- and p- nitrophenols by steam distillation.

(B). Separate a mixture of coloured organic compounds (fluorescein and methylene blue)

2 Experiment: - 2

To separate green leaf pigments by thin layer chromatography and determine their R_f values.

3 Experiment: - 3

(i). p-nitroacetanilide from acetanilide and its hydrolysis to p-nitroaniline.

(ii). Tribromobenzene from aniline

4 Experiment: - 4

Determination of : -

(a) Acid value: Resin & Plasticizers.

(b) Iodine number: Linseed Oil & Castor Oil.

(c) Saponification value: Coconut Oil & polyester

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ORGANIC CHEMISTRY

SYLLABUS

1. Laboratory Techniques

- (a) Steam distillation
Naphthalene from its suspension in water Separation of o- and p- nitrophenols.
- (b) Column Chromatography
Separation of fluorescein and methylene blue

2. Thin layer Chromatography

Determination of R_f values and identification of organic compounds.

- (a) Separation of green leaf pigments
- (b) Separation of a mixture of dyes using cyclohexane and ethyl acetate.

3. Synthesis of the following organic compounds

- (i). p-nitroacetanilide from acetanilide and its hydrolysis to p-nitroaniline.
- (ii). Tribromobenzene from aniline

4. Determination of : -

- (a) Acid value: Resin & Plasticizers.
- (b) Iodine number: Linseed Oil & Castrol Oil.
- (c) Saponification value: Coconut Oil & polyester.

EXPERIMENT: -1, (A). STEAM DISTILLATION

AIM: - To separate a mixture of O- and p- nitrophenols by steam distillation.

THEORY:-

The process is used for purification of those substances from non-volatile impurities which themselves are steam volatile but insoluble in water at the same time. For such a system, where two immiscible liquids are brought together which do not mix at all, each liquid behaves independently of the other and exerts a vapour pressure.

O-nitrophenol is steam volatile as it shows intramolecular hydrogen bonding and has a boiling point lower than p-nitrophenol which shows intermolecular hydrogen bonding. So they can be separated by steam distillation.



CHEMICAL USED:-

1. Ortho and para nitrophenol
2. 2% HCl solution
3. Powdered charcoal

APPARATUS USED:-

1. Round bottom flask
2. Sand bath
3. Filter paper
4. Buchner funnel
5. Condenser tube
6. Burner
7. Steam generator

PROCEDURE:-

1. Take the mixture of o-and p-nitrophenol in the round bottom flask.
2. While passing steam through it simultaneously, heat it on a sand bath, when ortho-nitrophenol passes over and para-isomer is left behind.
3. In ice cold water cool the distillate.
4. Filter at the pump and allow to drain.
5. Between folds of filter paper dry the compound and determine the melting point.
6. The removal of ortho-nitrophenol by steam distillation, cool the residue in the flask in ice for few minutes and filter.
7. For about five minute boil the crude para-nitrophenol, with 2% solution of HCl with a little powdered charcoal.
8. Through a Buchner funnel filter the solution and allow to cool gradually when colourless needle like crystal of para-nitrophenol separate out.
9. Dry them by pressing between folds of filter paper and note its melting point.
10. When ortho-nitrophenol solidifies in the condenser tube, stop passing cold water through the condenser.

PRECAUTION:-

1. All the apparatus used in experiment should be clean and dry.
2. Set all the apparatus properly.
3. Follow all the steps carefully.
4. To avoid the condensation of water, the round bottom flask is being simultaneously heated on a sand bath.

(B) COLUMN CHROMATOGRAPHY

AIM : - Separate a mixture of coloured organic compounds (fluorescein and methylene blue)

THEORY:-

In column chromatography, the stationary phase, a solid adsorbent, is placed a vertical glass, according to the amount of material which needs to be loaded on to the column, the columns may vary in sizes of their diameter and lengths. A plug of cotton wool is inserted in the neck of the tube.

Adsorbents used commonly for column chromatography are silica gel and alumina. The mobile phase, a liquid solvent is added to the top and flows down through the column by either gravity or external pressure.

As the solution passes through the column different components gets adsorbed to different extents at different level producing bands is selected a solvent or eluent is added, as soon as the last portion of the solution and enters the column. It acts as a moving phase. The selected solvent dissolve out the different components from the different zones selectively and thus work out the different band in the form of fraction which are collected separately. To get the components in pure form, the eluent from each fraction is then distilled off.

CHEMICAL USED:-

1. *Alumina 100-200 mesh*
2. *Methylene blue*
3. *Sodium salt of fluorescein*
4. *Rectified spirit*

APPARATUS USED:-

1. *Chromatography column*
2. *Test tube*
3. *Beaker*

PROCEDURE: -

1. Using rectified spirit prepare a slurry of 50-100 mg of activated alumina and pour this slurry into the column. On the top of the adsorbent leave a space of 4-5 cm for addition of solvent.
2. Mix solvent to the top of the adsorbent. The solvent flows slowly down the column. At the top level monitor the solvent, it does not go below the top of the adsorbent.
3. As the solvent close through the alumina and its level reaches the bottom of the column, the processes of pre-elution is complete and the column is ready to load.
4. In about 5 ml of rectified spirit dissolve 5 mg of methylene blue and sodium salt of fluorescein, the sample is ready to be loaded in the column. Only a few drops of solvent use to load the sample into the column.
5. Add rectified spirit, when the sample is introduced in the column to develop the chromatogram. After some time, blue colour of methylene blue travels down the column while fluorescein remains at the top.
6. Continue the process till whole of methylene blue has passed into the receiver placed below the column and the same becomes colourless.
7. Rectified spirit is placed by water has eluent solvent when fluorescein starts moving down the column. change the collection beaker and continue elution till whole of fluorescein is recovered.

PRECAUTIONS:-

1. To prevent the drying out of the column, the upper surface of the column always remains covered with the solvent throughout the experiment.
2. Eluents must be low boiling liquids show as to facilitate the quick removal from the fractions collected.

EXPERIMENT: -2, THIN LAYER CHROMATOGRAPHY

Determination of R_f values and identification of organic compounds.

AIM: - (a) To separate green leaf pigments by thin layer chromatography and determine their R_f values.

THEORY:-

In thin layer chromatography where the stationary phase is a polar adsorbent and the mobile phase can be a single solvent or combination of solvent. A thin layer of adsorbent usually silica gel or activated alumina on a smooth surface is used as a stationary phase and chromatogram is developed by upward capillary movement of the solvent through the thin layer of adsorbent.

In principle, the components will differ insolubility and in the strength of their adsorption to the adsorbent and some components will be carried farther up plate than others. When the solvent has reached the top of the plate, the plate is removed from the developing chamber, dried and separated components are visualized. If the compounds are coloured, visualization is straight forward. Various compound on the developed TLC plates are identify through their R_f values. R_f stands for Retention factor or Ratio of Fronts.

CHEMICAL USED:-

Chloroform

Acetone

Benzene

Silica gel

Spinach leaves

APPARATUS USED:-

Glass plates

Wide mouthed bottles with stoppers

Glass jars with lids

Tongs

Capillary tubes

Beaker

Filter paper

PROCEDURE:-

Slurry of adsorbent is prepared by mixing about 30 g of silica gel in small amount to 100 ml of chloroform in a wide mouth bottle with constant swirling motion; the bottles should be tightly stoppered. Similarly, we can also prepare slurry of alumina in alcohol.

By holding the two cleaned and dried glass plate together with tong, dipping them in the slurry of adsorbent and removing the plates quickly in this way, obtain a uniform coating of the slurry on one side of each plate. Do not dip the top and end of the plate now allow the slurry to drain and holding the top edges, separate the two plate and allow it to dry in air by placing these plate on a filter paper with slurry side upwards. These plates are known as chromatographic plates.

Prepare the 'extract of leaves' by dipping few crushed leaves of spinach in a little quantity of alcohol for 30 minutes in a beaker stirring with a glass rod the contents are filtered and the filtrate is taken as the 'extract of leaves'.

In the center of the chromatographic plate a drop of he green leaves extract is applied with the help of a capillary tube. Allow the drop dry in air.

In chromatography jar place the glass plate in such a way that it does not touch the side of the jar.

Into the chromatography jar lower the glass plate containing the developing solvent (alcohol and water in the ratio 1:1) care should be taken that the spot does not dip into the solvent and the glass strip hangs vertically straight. With the help of a lead cover the jar.

Remove the slide from the jar, when the developing solvent rises on a certain suitable distance and dry it in air.

Mark the level of the liquid with a pencil.

Mark the central points of each support.

The distance travelled by the spots and the developing agent to be measured.

Calculate the R_f values of the spots corresponding to different components.

By using the relation

$$R_f = \frac{\text{Distance travelled by the component}}{\text{Distance travelled by the solvent}}$$

CALCULATION: -

Distance travelled by component 1 = d_1 m

Distance travelled by component 2 = d_2 m

Distance travelled by component 3 = d_3 m

Distance travelled by developing liquid = d_4 cm

Rf value of component 1 = d_1/d_4

Rf value of component 2 = d_2/d_4

Rf value of component 3 = d_3/d_4

PRECAUTION: -

The glass plate used must be thoroughly clean and dry. Fine capillary tube should be used for applying a spot of solution. The glass plate should be kept erect. During the experiment, keep the glass jar always covered and undisturbed. The slurry bottle must be tightly stoppered. The spots of the solution must not dip in the developing solvent.

EXPERIMENT: – 3, (i). p-nitroacetanilide from acetanilide and its hydrolysis to p-nitroaniline

Principle:

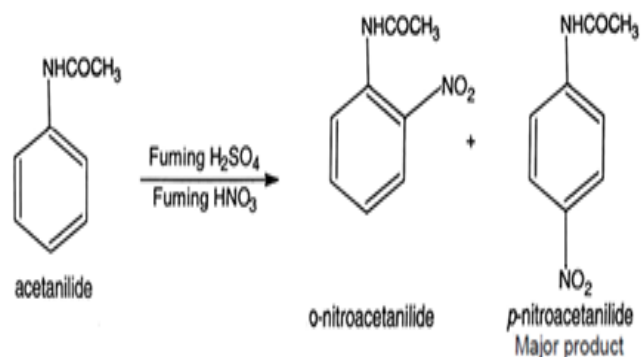
Here in first step electrophilic aromatic substitution (nitration) of nitronium ion is occurring towards para position of acetanilide more than ortho position due to steric reasons. Nitronium ion is the electrophile generates from fuming nitric acid in presence of fuming sulphuric acid. In the second step p-nitroaniline is prepared from p-nitro acetanilide due to hydrolysis of acetate ion from acetamido functional group in presence of concentrated sulphuric acid.¹

Aim:

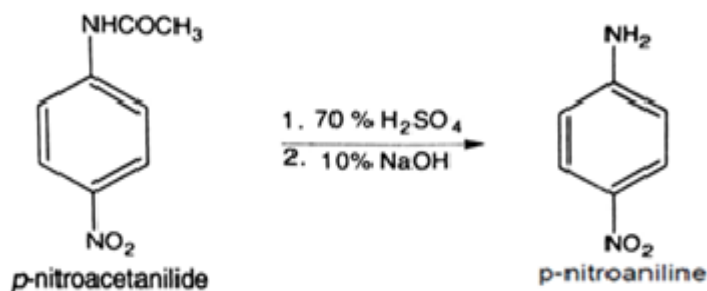
To prepare p-nitro aniline from acetanilide.

Reaction:

Step 1:

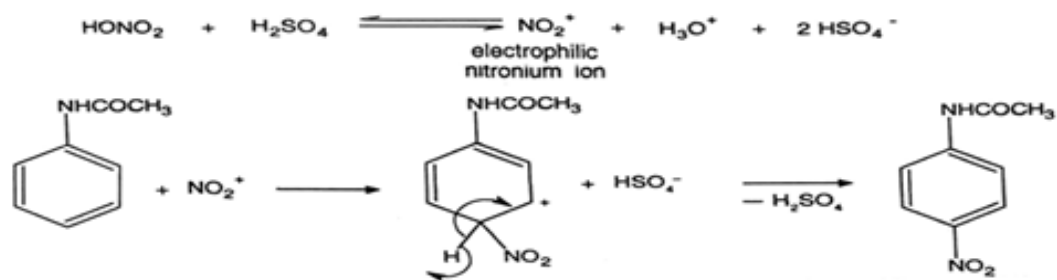


Step 2:

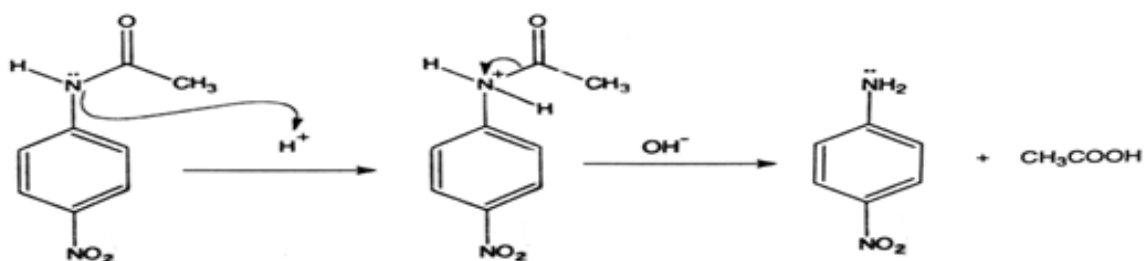


Mechanism: -

Step 1:



Step 2:



Use:

It is an intermediate for dyes, pigments, pharmaceuticals (Paracetamol, Phenacetin etc.), pesticides and rubber chemicals.

REQUIREMENTS

Chemicals: Acetanilide

Glacial acetic acid

Conc. HNO₃

Conc. H₂SO₄

70% H₂SO₄ solution

10% NaOH solution

Apparatus: Conical flask

Beaker

Pipette

Glass rod

Buchner funnel

PROCEDURE

Step 1: Preparation of p-Nitroacetanilide from Acetanilide.

Add dry acetanilide (25 g) to glacial acetic acid (25 ml) in a beaker and then introduce concentrated sulphuric acid (50 ml) slowly with constant stirring to obtain clear solution. Place the beaker in a freezing mixture of ice and salt to cool the solution below 5 °C. Add a cold mixture of concentrated nitric acid (11 ml) and concentrated sulphuric acid (7 ml) drop wise with constant stirring to a reaction mixture while maintaining the temperature below 5 °C. After adding all the mixed acid, remove the beaker from the freezing mixture and keep it for 1 hr at room temperature. Pour the reaction mixture into an ice cold water (30 ml) to obtain the crude product of p-nitroacetanilide. Filter it on suction, wash with cold water till free from acid and recrystallize the pale yellow product from ethanol to get colourless crystalline solid, m.p. 214.5 °C.

Note:

o-Nitroacetanilide remains in the filtrate due to its high solubility in water.

Step 2: Preparation of p-Nitroaniline from p-Nitroacetanilide.

Place 30 g of p-nitroacetanilide and 150 ml of 70% H₂SO₄ (prepared by adding 100 ml conc. acid to 75 ml water carefully) in a round-bottomed flask. Reflux the mixture for 20-30 min. and pour the hot solution into 1000 ml of cold water. Neutralize with 10% NaOH, cool and filter the yellow crystalline product on a Buchner funnel. Wash it thoroughly with water. Recrystallize the product using a mixture of equal volume of rectified spirit and water or from hot water.²

Calculation:

Here limiting reagent is acetanilide; hence yield should be calculated from its amount.

Molecular formula of acetanilide = C₈H₉NO

Molecular formula of p-nitroaniline = C₆H₆N₂O₂

Molecular weight of acetanilide = 135 g/mole

Molecular weight of p-nitroaniline = 138 g/mole

Theoretical yield:

135 g acetanilide forms 138 g p-nitroaniline

Therefore, 25 g acetanilide will form? (X) g p-nitroaniline

$$X = (138 \times 25) / 135 = 25.55 \text{ g}$$

Theoretical yield = 25.55 g

Practical yield = _____ g

$$\% \text{ Yield} = (\text{Practical Yield}) / (\text{Theoretical Yield}) \times 100$$

CONCLUSION

p-nitroaniline was synthesized and the percentage yield was found to be.....%

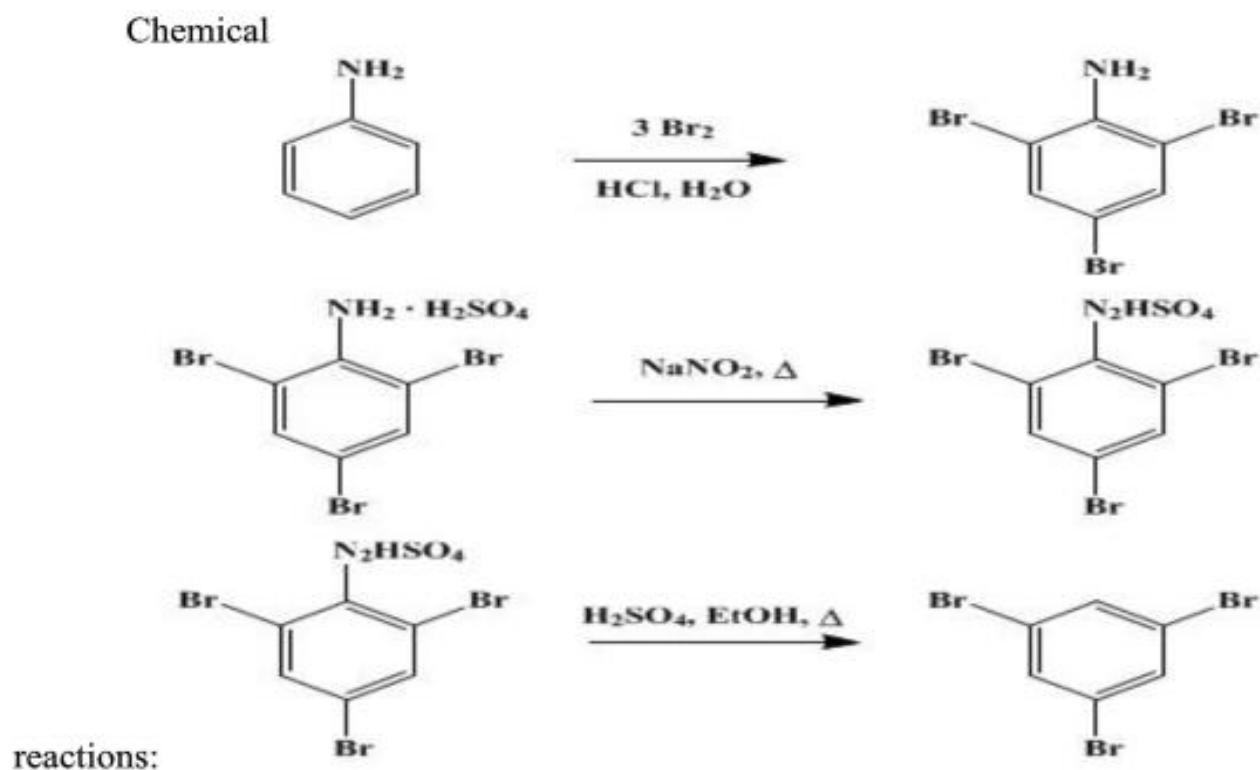
EXPERIMENT: - 3, (ii). 1,3,5 - TRIBROMOBENZENE FROM ANALINE

AIM: - To prepare tribromobenzene from aniline.

REQUIREMENTS: -

Chemicals required: - Aniline, Bromine, Hydrochloric acid, Sodium nitrite Benzene, activated animal charcoal.

Apparatus required: - Conical flask, beaker, glassrod, measuring cylinder, Buchner funnel.



Procedure:

The apparatus consist sofa 12-l.round – bottomed flaskanda 250 - cc.suction flask provided

With stoppers and glasstubes. After the flask shave been charged, Aissurrounded byanice

Bath and Bbya water bath heated to 40–50°. In flask Aare placed 100g. (1.1moles) Of aniline, 1l. of water, and 100cc. (1.2moles) of concentrated hydrochloricacid; when

The anilinehas dissolved,

Sufficient water is added to bring the volume of the solution to 5l.

Flask Bischarged with 577g. (185cc.,3.6moles) of bromine; the baths area djusted, and a Rapid stream fair saturated with bromine vapor is drawn into Aby applying suctionat C



The introduction of bromine is continued until the solution assumes a distinctly yellow color; approximately three to four hours is required, and the reaction is then complete. The tribromo aniline is filtered on a Büchner funnel, thoroughly washed with water to remove hydrobromic acid, sucked as dry as possible, and taken without further drying to the next step. The moist tribromo aniline, together with 2.11 of 95 percent alcohol and 525 cc. of benzene, is placed in a 5-l. two-necked flask. One neck of the flask bears a reflux condenser; the other is closed with a stopper that can be removed momentarily for the addition of reagents. The tribromo aniline is brought into solution by heating the flask on a steam bath. To this solution is added 140 cc. of concentrated sulfuric acid, and then 140 g. (2.03 moles) of powdered sodium nitrite as rapidly as the violence of the reaction will permit. When the action has moderated, the solution is brought to boiling and maintained there as long as gas is evolved. It is then allowed to stand in a warm place for three hours more. After the mixture has been cooled (preferably in an ice bath), the mother liquor is removed from the solid by decantation through a Büchner funnel; if necessary, a wire gauze is used to retain the solid in the flask. To the solid is then added a solution of 150 cc. of concentrated sulfuric acid in 1.5 l. of water. When the excess sodium nitrite has

been decomposed, the solid is transferred to a Büchner funnel and washed first with water and then with a small amount of alcohol. The yield of crude, dry tribromo benzene is 250–260g. (74–77 percent of the theoretical amount). The product is reddish brown and melts at 112–116°. For further purification, 100g. of the crude product is dissolved in a boiling mixture of 1560cc. of glacial acetic acid and 350cc. of water; the solution is boiled for a few minutes with 25g. of decolorizing carbon, filtered red hot, and allowed to cool. The crystals are washed on a Büchner funnel with a small amount of chilled 95 percent alcohol to remove the acetic acid.

The whole of the crude product crystallized in this way furnishes 216–240g. (64–71 percent of the theoretical amount) of slightly colored tribromo benzene, melting at 121.5–122.5° (corr.).

The yield of crude product may be increased 50–60g.— making a total of 300–320g. (89–95 percent of the theoretical amount)— by working up the mother liquors. The alcoholic liquors and washings are diluted to 6l., and the aqueous layer is separated from the heavy oil at the bottom. The benzene is then distilled from the oil in a distilling flask, care being taken not to overheat the product after the benzene has been removed. The

Residual tribromo benzene may be recrystallized in the manner described above.

2. Notes

1. Considerable heat is generated by the reaction, and it is necessary to cool A to prevent the loss of bromine. Flask B must be kept at 40–50° to ensure a high concentration of bromine vapor in the airstream; the violent agitation produced by air saturated at lower temperatures whips the reaction mixture into a light foam that is likely to overflow the flask.

3. It is well to interpose a safety flask between the reaction flask and the aspirator. The safety flask is partially filled with water through which the air stream passes after the bromine vapor has been removed. This device has a double purpose; it enables one to gauge the rate of aspiration by the rate at which the air current bubbles through the water, and it serves to detect the escape of bromine vapors from the reaction flask. A small amount of bromine will impart to the water a distinctly yellow color. In case bromine does escape into this trap a momentary release of the suction will cause the wash water to be drawn into the reaction flask, thus returning the bromine to the reaction mixture.

EXPERIMENT: – 4 (a) Determination of Acid value of Resin

Acid Value Determination in Powder Coating Resins

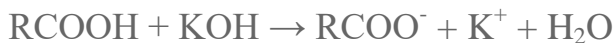
This application describes the determination of the acid value, expressed in mg KOH/g resin, of powder coating resins by potentiometric titration.

Background:

Powder coating is nowadays a widely applied technique that shows remarkable advantages over other coating processes. Even thick coatings can be produced without the need of solvents or finishers that are costly, and are often detrimental to the environment. The basis of the powder coating formulation is the resin. Depending on the application, the resin has to satisfy different requirements. Hence, there is a large variety of resins available. The properties of these resins are mainly affected by the sort and number of functional groups on the resin.

One important characteristic of the powder coating resins is the total number of acidic functional groups (i.e. carboxyl groups). Mainly relevant here are free carboxyl groups as well as anhydrides, which are hydrolyzed to carboxyl groups during the analysis. The so-called acid value (AV) represents the amount of mg KOH/g sample that is consumed for the neutralization of the acidic groups contained in the sample. Anhydrides add double to the acid value as they react with water to form two equivalents of carboxylic acids.

Neutralization of carboxyl groups:



Hydrolyzation of anhydrides



**(b). IODINE NUMBER OF LINSEED AND PETROLEUM
(CASTROL) OILS**

INTRODUCTION

The linseed oil used in the manufacture of printing ink is of the so-called “burnt” type. There are two general processes for its manufacture—one in which the oil is heated until the vapors take fire and continue to burn, the oil being allowed to burn until it attains the desired viscosity; and a second in which the oil is heated without permitting it to take fire.

Burnt oil is prepared in several grades, all differing from the raw oil in an increase of viscosity, specific gravity, and acid number, and a decrease in the iodine number. The longer the oil is heated the greater these differences become.

In the determination of the iodine value of some burnt linseed oils difficulty was experienced in obtaining concordant results. Leeds has published some figures for iodine absorption of litho-graphic oils. Kitt has also published a series of results which show decrease in iodine absorption with increasing viscosity, but the figures do not agree with those given by Leeds. These determinations were made according to the Hiibl method. In recent years this method has been so generally replaced by the rapid and more convenient Hanus method that it was considered desirable to learn the values afforded by this method. Preliminary work developed the fact that small variations in the method employed produced varying results. It has been well established that iodine absorption includes not only the halogen taken up by unsaturated compounds, but that the substitution of halogen for hydrogen with the formation of halogen acid occurs simultaneously. Various methods for determining the amount of substitution have been suggested, with the view of obtaining a corrected value which would represent the addition only, but very little use has been made of them. It has been generally recognized that an excess of iodine is necessary, and it has been assumed that all oils are alike in the amount of excess required. The appended results obtained by us show that this is far from being true. Various quantities of iodine have been suggested as to the proper excess which should be present, but practically all of these are given in connection with discussions of the Hiibl method, and may not hold true for the Hanus method. Furthermore, there has been a lack of uniformity in the use of the term “excess of iodine.” In the tables which follow whenever this expression is used it shall be understood to mean that percentage of the total amount added which remains unchanged at the expiration of the time allowed for absorption.

PROCEDURE: -

The method employed was essentially the modification of the Hanus method suggested by Hunt. Thirteen and two-tenths grams of iodine were dissolved in liter of glacial acetic acid (99.9 per cent), and 5 cc of bromine added. This solution was always allowed to stand for some days before being used. The thiosulphate solution employed was approximately tenth normal, and was standardized by means of potassium bichromate. Standardization was repeated at frequent intervals. A freshly prepared starch solution was used; also a 10 per cent solution of potassium iodide, prepared in small amounts and kept in a brown bottle. The temperature of the room was maintained at 25° to prevent variations caused by change of temperature. The reagents were of standard quality and errors caused by impurities were eliminated by running blanks with each series of determinations. When it was desired to use like amounts of an oil in a series of tests, 4 grams of the oil were dissolved in chloroform in a 200-cc graduated flask and the solution allowed to reach room temperature. Ten-cc portions of this solution, representing 0.2 gram of oil, were measured from a burette. The general procedure was as follows: -

The exact weight of oil was transferred to 200 to 300-cc glass-stoppered Jena bottles; the required amount of Hanus solution was added from a burette. The mixture was allowed to stand for exactly 30 minutes in a dark closet; 23 cc of the 10 per cent potassium iodide solution and 100 cc of water were added, and the excess of iodine was immediately titrated with thiosulphate.

The factors known to influence the iodine number are the temperature, the time of absorption, the weight of oil taken, and the excess of iodine present obtained by increasing the amount of iodine solution. The exact effect of each factor was studied by varying one at a time, this procedure being followed with a series of oils.

CONCLUSION: -

A study was made of the effect of temperature on the iodine absorption, from which it would appear that it may be feasible to improve the Hanus method by working at lower temperatures than those which have been used, and thus eliminate part of the substitution which occurs simultaneously with the addition.

The results obtained show that when used under exact conditions the Hanus method is to be recommended for simplicity of preparation of the solutions employed, ease of manipulation, and for concordance of results obtained.

Suggestions are made for the standardization of the method of determining the iodine number of mineral oils. The necessity for such action is shown by the results on several samples of lubricating oils.

We wish to express our appreciation of the courtesy of Messrs. Anlt & Wiborg, of Cincinnati, Ohio, in furnishing us with the burnt linseed oils used in this work.

(c). SAPONIFICATION VALUE OF COCONUT OIL

BACKGROUND

The number of milligrams of KOH required to neutralize the fatty acids resulting from the complete hydrolysis of 1g of fat is called as saponification value.¹ This value is used for comparison of the fatty acid chain length in oils.

Saponification is the process in which fatty acid esters are hydrolyzed to glycerol and potassium salts of fatty acids on refluxing with alkali.

REQUIREMENTS

Beaker

Weighing balance

Fat solvent (equal volumes of 95% ethanol and ether)

Alcoholic KOH

0.5N HCl

Reflux condenser

Phenolphthalein

Coconut oil

PROCEDURE

Alcoholic KOH

Dissolve 40 g KOH in 1.0 L alcohol maintaining the temperature at or below 15° C, mix well to dissolve.

Take a suitable glass container and put it over a weighing balance tared to zero. Weight 1.0 g of fat (butter or coconut oil) and put it in the beaker with 3 ml of fat solvent, mix well. Add the above mixture with 25 ml of alcoholic KOH and mix well, arrange this mixture with a reflux condenser.

Repeat the above process & reflux condenser arrangement excluding fat.

Keep both the arrangements in a boiling water bath for about 30 minutes. Wait to cool. Add phenolphthalein indicator to both the flasks and titrate with 0.5N HCl. The endpoint of test and blank (excluding fat) is to be recorded, the difference of which tells the volume of KOH consumed by 1 g of fat.

CONCLUSION

The saponification value of various fats like ghee, olive oil, sesame oil, butter etc can be determined with above process and formula.