Komar University of Science and Technology (KUST)

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ORGANIC CHEMISTRY

CHM 2415L
Laboratory Manual
Medical Laboratory Science Department

Prepared by
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Preface and Acknowledgments

In preparing this laboratory manual, I am thankful to the efforts of KUST administration, Medical Laboratory Science Department, and staff members who made this edition possible to be completed with their support.

This laboratory manual shares the outline and pedagogical philosophy of the textbook, *Organic Chemistry, Ed, David Klein. Johns Hopkins University, John Wiley and Sons, Inc. 2015*, as in previous editions, CHM 1410C and 2411C we have strived for the clearest possible writing in the procedures. The experiments give the student a meaningful, reliable laboratory experience that consistently works, while covering the basic principles of general chemistry, organic, and biochemistry.

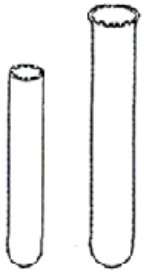
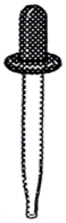
Three basic goals were followed in all the experiments: (1) the experiments should illustrate the concepts learned in the classroom; (2) the experiments should be clearly and concisely written so that students will easily understand the task at hand, will work with minimal supervision because the manual provides enough information on experimental procedures, and will be able to perform the experiments in a two-and-a-half-hour laboratory period; (3) the experiments should not only be simple demonstrations, but also should contain a sense of discovery.

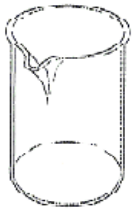
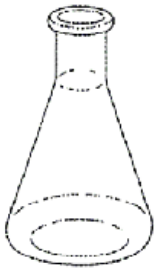
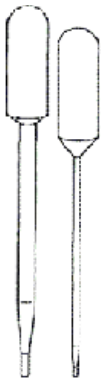
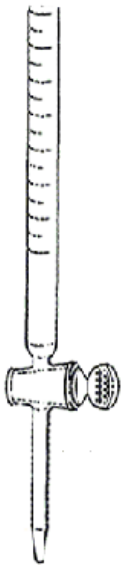
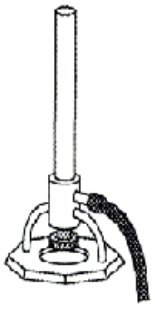
I anticipate that students will find it inspiring in studying different aspects of chemistry.

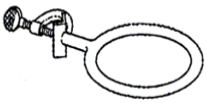
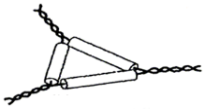
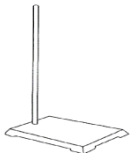
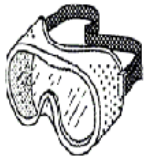
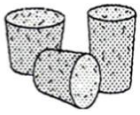
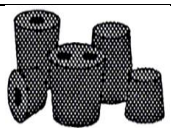
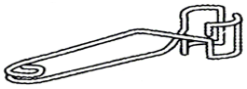
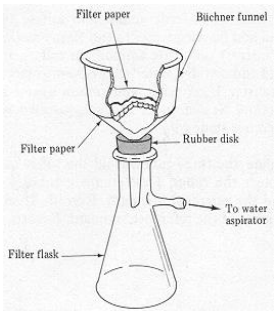
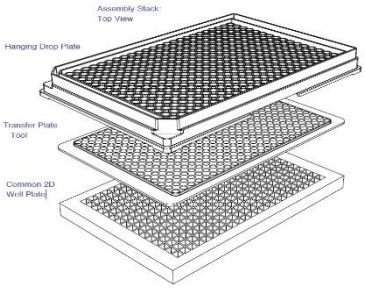
Contents

1. Common Laboratory Apparatus*

Laboratory apparatus and equipment like electronic scales, glassware, burners, graduated cylinders, and more, help the scientist conduct experiments, observe data, heat liquids, make measurements, and more.

Items Name	Picture	Items Name	Picture
Volumetric Flask		Test Tubes	
Watch Glass		Crucible and lid	
Dropper		Evaporating dish	
Funnel		Graduated cylinder	

Items Name	Picture	Items Name	Picture
Mortar and pestle		Beaker	
Stirring rod		Erlenmeyer Flask	
Micropipette		Burette	
Thermometer		Bunsen burner	

Items Name	Picture	Items Name	Picture
Wire gauze		Clamp	
Iron ring		Clay triangle	
Stand		Tong	
Wash bottle		Safety goggles	
Corks		Rubber Stopper	
Brush		Forceps	
Test tube holder		Spatulas	
Suction Filteration		Well plate	

Items Name	Picture	Items Name	Picture
Centrifuge		Oven	
Water bath		Electronic Lab Scale	
Hot plate		pH-meter	

* Matin J., Martin C. (2012). Catalyst: the Pearson Custom Library for Chemistry: Laboratory Experiments, Pearson Prentice Hall. ISBN13: 978-0-536-93404-8

2. Chemistry Lab Safety

The chemistry laboratory can be a place of discovery and learning. However, by the very nature of laboratory work, it can be a place of danger if proper common-sense precautions aren't taken. While every effort has been made to eliminate the use of explosive, highly toxic, and carcinogenic substances from the experiments which you will perform, there is a certain unavoidable hazard associated with the use of a variety of chemicals and glassware. You are expected to learn and adhere to the following general safety guidelines to ensure a safe laboratory environment for both yourself and the people you may be working near. **Each student will have to pass a lab safety exam before conducting any lab work to evaluate his/her understanding to the lab rules and safety precautions. Students who fail in that exam will have to take orientation session about lab safety before they can start lab work.** Additional safety precautions will be announced in class prior to experiments where a potential danger exists. **Students who fail to follow all the safety rules may be asked to leave the lab or suffer grading penalties.**

2.1 Laboratory Attire

1. Safety goggles *must be worn at all times* while in the laboratory. This rule must be followed whether you are actually working on an experiment or simply writing in your lab notebook. You must wear safety goggles provided by the chemistry department.

2. Contact lenses are not allowed. Even when worn under safety goggles, various fumes may accumulate under the lens and cause serious injuries or blindness.

3. Closed toe shoes and long pants must be worn in the lab. Sandals and shorts are not allowed.

4. Long hair must be tied back.

2.2 Laboratory Conduct

1. Eating, drinking, and smoking are strictly prohibited in the laboratory.

2. No unauthorized experiments are to be performed. If you are curious about trying a procedure not covered in the experimental procedure, consult with your laboratory instructor.

3. Coats, backpacks, etc., should not be left on the lab benches and stools. Beware that lab chemicals can destroy personal possessions.

4. Always wash your hands before leaving lab.

5. Be especially careful of spills around the balance. These electronic devices are *extremely* sensitive to corrosion. A brush is kept near the balance so you can brush the balance thoroughly after *each* use.

6. Clean any spill near the balance *immediately* and report it to instructor.

7. Notify the instructor immediately in case of an accident.

8. Consider *all* chemicals to be hazardous as a result never taste anything, never directly smell the source of any vapor or gases, by means of your couple hands, bring a small sample to your nose, Fig 2.2.1

Fig 2.2.1 Waft Toward Your Nose

9. Know what chemicals you are using. Carefully read the label *twice* before taking anything from a bottle.

10. Excess reagents are **never** to be returned to stock bottles. If you take too much, dispose of the excess.

11. Many common reagents, for example, alcohols and acetone, are highly flammable. *Do not use them anywhere near open flames.*

12. Always pour acids into water. If you pour water into acid, the heat of reaction will cause the water to explode into steam, sometimes violently, and the acid will splatter.

13. If chemicals come into contact with your skin or eyes, *flush immediately* with copious amounts of water and consult with your instructor.

14. Never point a test tube or any vessel that you are heating at yourself or your neighbor, Fig. 2.2.2.

15. Dispose of chemicals properly. Waste containers will be provided and their use will be explained by your TA. Unless you are explicitly told otherwise, assume that only water may be put in the lab sinks.

16. Clean up all broken glassware immediately and dispose of the broken glass properly.

Fig. 2.2.2 Pointing a Test Tube at Your Neighbor

17. Never leave burners unattended. Turn them off whenever you leave your workstation. Be sure that the gas is shut off at the bench rack when you leave the lab.

18. Beware of hot glass--it looks exactly like cold glass.

2.3 Fire Case

1. In the event of fire, *do not panic*.

2. If a small portion of your clothes catches fire, the fire may be extinguished by patting it out.

3. *Never* use a fire extinguisher on a person. Carbon dioxide fire extinguisher is extremely cold and may cause shock to the person, frostbites, or harm to the eyes.

4. If a fire should occur in a breaker or some other container, cover it with a glass dish or other flame-retardant item.

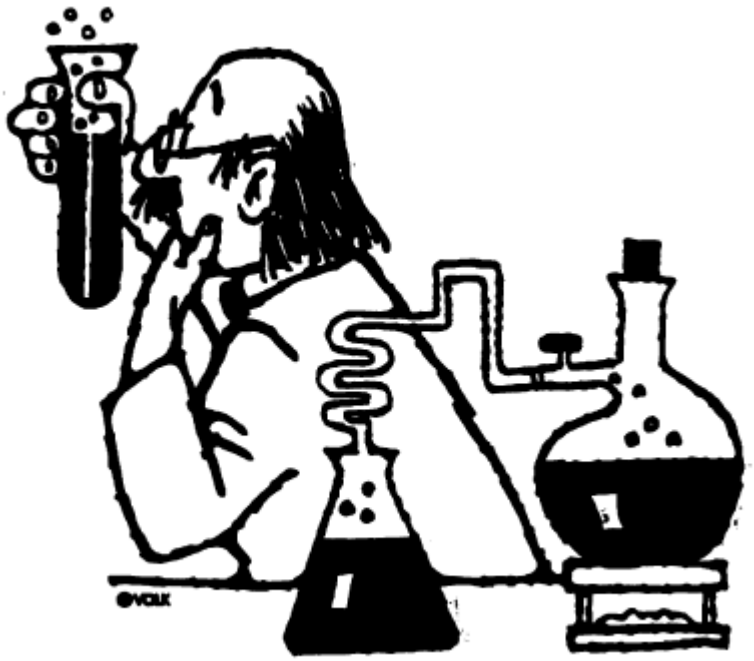
3. Writing Lab Reports

The lab report for this course will be represented by a designed report sheet prepared by the course instructor and accompanying with each experiment. This sheet will illustrate the understanding of the experiment via answering some questions related to the work and it also summarize the data via tables with simple calculations whenever it is needed.

4. Submission of Lab Reports

The lab report sheet of previous week experiment should be submitted to the lab assistant or the course instructor at the beginning of the next experiment lab work. Late submission will cause deduction 1 grade of your total report sheet grade for each one hour lateness after the submission due date that announced by the instructor. No report sheets will be collected after 10 hours from the submission date. Also individual submission is not accepted, each group should submit one report sheet for their work.

5. Laboratory Experiments



Experiment 1

S_N1 Synthesis of tert-Butyl Chloride

Objectives

1. Synthesize *tert*-butyl chloride via an S_N1 reaction.
2. Confirm the presence of a tertiary alkyl halide using the silver nitrate test.

Introduction

Alkyl halides can be prepared from their corresponding alcohols via an acid catalyzed substitution reaction. The mechanism of these acid catalyzed substitution reactions are labeled as S_N1 (substitution, nucleophilic, unimolecular) and S_N2 (substitution, nucleophilic, bimolecular). Tertiary alcohols follow the S_N1 route, primary alcohols follow the S_N2 route, and secondary alcohols can follow either path. Under acidic conditions, the mechanism (*Figure 1.1*) of the S_N1 reaction involves rapid protonation of the alcohol, followed by the loss of water as the rate-determining step. This generates a relatively stable carbocation which is then attacked by the halide ion to form the alkyl halide. S_N1 reactions are favored by tertiary alcohols because they tend to form more stable carbocations.

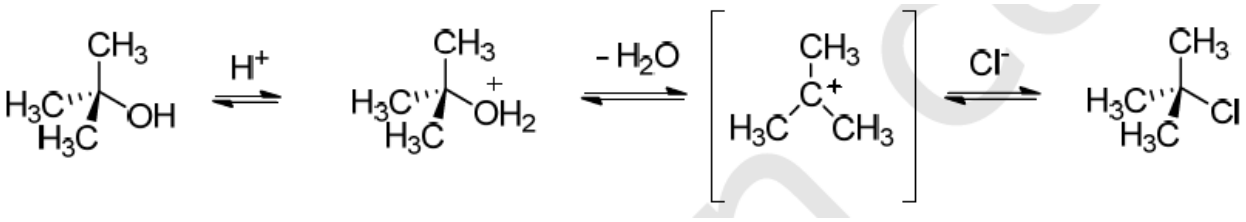


Figure 1.1: Conversion of t*-butanol into* t*-butyl chloride by an S_N1 mechanism*

Confirmation of your product, *tert*-butyl chloride, can be performed by reacting the product with a solution of silver nitrate in ethanol. The tertiary alkyl halide will react by means of an S_N1 mechanism with the silver nitrate to form an insoluble silver halide. The appearance of a precipitate indicates a positive result indicating the presence of a tertiary alkyl halide.

Materials

Table 1.1: Chemicals and supplies

Chemicals	Supplies
concentrated HCl	25 mL graduated cylinder
tert-Butanol, reagent grade	10 mL glass vials and caps (2)
Saturated $NaHCO_3$ solution	10 mL graduated cylinder
Saturated NaCl solution	50 mL beaker
Na_2SO_4, anhydrous	250 mL beaker
DI water	125 mL Erlenmeyer flask
Ice	Separatory funnel apparatus
1% ethanolic-$AgNO_3$ solution	Glass funnel
tert-Butyl chloride (product from Part I)	Disposable droppers
	3 test tubes
	Test tube rack

Procedure

Part I: Synthesis of *tert*-butyl chloride

Note: Conduct step 2-3 in a fume hood and leave it inside the hood until you finish setting up the Separatory funnel apparatus in step 4.

1. Prepare an ice-water bath in a 250 mL beaker.

2. Measure 15 mL of concentrated HCl using a graduated cylinder and carefully transfer to a 50 mL Erlenmeyer flask.

   CAUTION: Handle the HCl with care. It can cause painful burns if it comes in contact with the skin.

3. Cool the Erlenmeyer flask containing the acid in the ice-water bath that you prepared in step 1.

4. Set up the reaction in a Separatory funnel. *Figure 1.2*:

   a. Clamp the support ring onto a ring stand, place the Separatory funnel into the ring, and insert a funnel.
   b. Carefully transfer the cooled acid to the Separatory funnel.
   c. Weigh 4.0 g of *tert*-butanol using a beaker and transfer to the Separatory funnel. Record the mass in the report sheet.

Figure 1.2: Separatory Funnel Set up

5. Swirl the solution in the Separatory funnel for five minutes.

   Note: ***Leave the funnel uncapped as you swirl to allow the gases to escape.***

6. Cap the Separatory funnel, invert it, and immediately open the stopcock to release the pressure. *Figure 1.3.*

   Note: ***When venting the funnel, point the tip in a direction where there's no one and open the stopcock to release the pressure.***

7. Shake the funnel for 15 minutes. Vent occasionally to prevent pressure buildup.

8. Place the funnel in the support ring and allow the solvent and aqueous layers to separate. Leave the funnel uncapped.

9. Drain the lower aqueous layer into a 125 mL Erlenmeyer flask. Be sure to label the flask as **waste**.

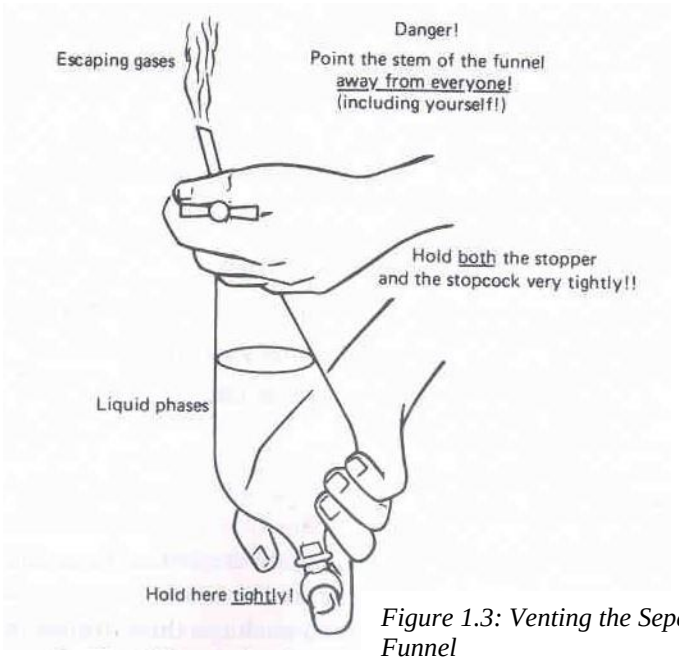


Figure 1.3: Venting the Separatory Funnel

10. Prepare to wash the solution:
    a. Wash the organic layer with 30 mL of a saturated sodium bicarbonate solution. Add the sodium bicarbonate solution slowly. Swirl the funnel several times until the bubbling has stopped.
    b. Cap the funnel, invert, shake, and immediately vent.
    c. Place the uncapped funnel in the support ring and allow the layers to separate.
    d. Drain the aqueous layer into the waste beaker.

11. Repeat Step 10 with using another 30 mL of a saturated sodium chloride solution.

12. Prepare to dry the product:
    a. Place a pea-sized amount of anhydrous sodium sulfate in a 10 mL vial.
    b. Transfer the organic layer into the vial and swirl until clumps form.
    c. Cap the vial and wait five minutes to allow the sodium sulfate to dry the product.
13. While waiting, weigh a clean and dry 10 mL vial. Record the mass in your report sheet.

14. Carefully pipet the product into the clean vial and weigh. Be careful not to transfer any of the sodium sulfate. Record the mass in your report sheet.

Part II Silver Nitrate Test for Tertiary Alkyl Halides

1. Set up the reaction.
   a. Obtain two test tubes.
   b. Pipet 10 drops of distilled water into test tube 1.
   c. Pipet 10 drops of your product into test tube 2.

2. Add approximately 1 mL of the 1% ethanolic silver nitrate solution to each test tube. Swirl each test tube to mix the contents. The appearance of a white precipitate indicates the presence of a 3° halide. Record your observations in the report sheet.

   Note: Avoid getting the $AgNO_3$ solution on your skin.

Report Sheet

Grade

Group Names:

Date:

1. Collecting Data:

Table 1.2: Part I: Synthesis of tert-Butyl Chloride

Mass of tert-Butanol	g
Mass of vial	g
Mass of vial and product	g
Mass of product	g
Theoretical mass of tert-Butyl Chloride	g
Percent yield	%

Table 1.3: Part II: Silver nitrate test for alkyl halides

Test	Compound	Observation
1	H_2O	
2	Product	

2. Answer the following questions:
   a. Write an S_N1 mechanism of the reaction?

   b. Why it is necessary to conduct the reaction under acidic conditions?

   c. Write the molecular chemical equation for the reaction between alcohol and HCl.

Experiment 2

Nucleophilic Substitution Reaction: S_N1 versus S_N2

Objectives

1. Learn how variation in organo-halide structure affects the rate of S_N1 and S_N2 reactions
2. Observe the reaction rate by measuring the time required for a visible change to occur (formation of a precipitate).

Introduction

Nucleophilic substitution is one of the most useful and well-studied class of organic reactions. These reactions can occur by a range of mechanisms. S_N2 and S_N1 are the extremes.

The S_N2 reaction occurs in a single step. The nucleophile enters as the leaving group — usually a halide ion — departs. The reaction displays second-order kinetics; its rate is proportional to the concentration of the organo-halide *and the nucleophile*.

In the SN1 reaction loss of the leaving group occurs first to generate a carbocation intermediate. The carbocation then captures a nucleophile, often the solvent (followed by proton transfer to produce the final neutral product). In this case the reaction is called a *solvolysis*.

Because the first step is rate-determining, the S_N1 reaction displays first-order kinetics; its rate depends *only* on the concentration of the organo-halide. It will be easier to remember which label goes with which mechanism if you associate the "***1***" in S_N1 with ***carbocation*** rather than with the kinetic order of the reaction. (Perhaps this should be called S_NCarbocat or S_NC+.)

Which mechanism occurs under a certain set of conditions and how fast it occurs depend on a variety of factors. The structure of the organo-halide, the leaving group, the nucleophile, and the solvent can all play a role.

An assortment of alkyl, alkenyl, and aromatic chlorides and bromides will be available.

To encourage an S_N2 reaction mechanism you will use a solution of NaI in acetone. Iodide is a good nucleophile, and if it displaces bromide or chloride, NaBr or NaCl will precipitate (these are much less soluble in acetone than NaI). To encourage an S_N1 reaction mechanism you will use a solution of $AgNO_3$ in ethanol. Ethanol is a polar protic solvent and can promote ionization of certain organo-halides. If halide ion is released a precipitate of AgCl or AgBr will form.

Materials

Table 2.1: Chemicals and supplies

Chemicals	Supplies
1 M NaI/acetone	Test tube (7)
1-bromobutane	10 mL Graduated cylinder (2)
bromobenzene	Disposable droppers
2-bromo-2-methylpropane	Warm water bath 40-50°C
Allyl bromide	
Allyl chloride	
Bromo benzene	
Benzyl chloride	
2% $AgNO_3$/EtOH	

Note: The TA should prepare necessary solutions before lab starts.

Procedure

Part I: S_N2 Reaction

1. Bring 3 small, dry, clean test tubes and add to each test tube 4 drops of 1-bromobutane, bromobenzene, 2-bromo-2-methylpropane respectively.

2. Add 1 mL of NaI/acetone to each test tube and record the time for precipitation to form in table 2.1 in the report sheet.

3. If no precipitate form after about 3 minutes, warm the test tubes to 40-50°C and record the time required for precipitation in table 2.1. You will call a test tube (unreactive) if no reaction visible after about 10 minutes.

4. Complete table 2.1 based on what you leaned in class.

5. Bring 4 small, dry, clean test tubes and add to each test tube 4 drops of the allyl and bezyl halides that's available in the lab and repeat steps 2-5. *see the note*

   Note: the allyl and benzyl halides should be tested in the hood because they are strong lachrymators.

Part II: S_N1 Reaction

Repeat step 1-5 using $AgNO_3$/EtOH solution and record your observation in table 2.3

Report Sheet

Date:

Grade

Group Names:

Table 2.2: Recording observation for S_N2 reaction

Structure of organo-halide		Adding 1 M NaI/acetone	*Predict the relative reactivity	Observed time for forming ppt
1-bromobutane				
2-bromobutane				
2-bromo-2-methylpropane				
Allyl bromide				
Allyl chloride				
Bromo benzene				
Benzyl chloride				

*use the words: very slow, slow, fast, very fast…etc

Report Sheet 2

Table 2.3: Recording observation for S_N1 reaction

organo-halide	Structure of organo-halide	Adding 2% $AgNO_3$/EtOH	*Predict the relative reactivity	Observed time for forming ppt
1-bromobutane				
bromobenzene				
2-bromo-2-methylpropane				
Allyl bromide				
Allyl chloride				
Bromo benzene				
Benzyl chloride				

*use the words: very slow, slow, fast, very fast...etc

Report Sheet 3

Answer the following questions:

1. What was the effect of substitution at the C undergoing nucleophilic attack, in particular, 1° vs 2° vs 3° alkyl?

2. Do the allylic and bezylic halides fit this pattern? If not, suggest an explanation.

3. What was the effect (if any) of changing the leaving group?

Experiment 3

Identification of Alcohols and Phenols

Objectives

1. To learn characteristic chemical reactions of alcohols and phenols.
2. To use these chemical characteristics for identification of an organic compound.

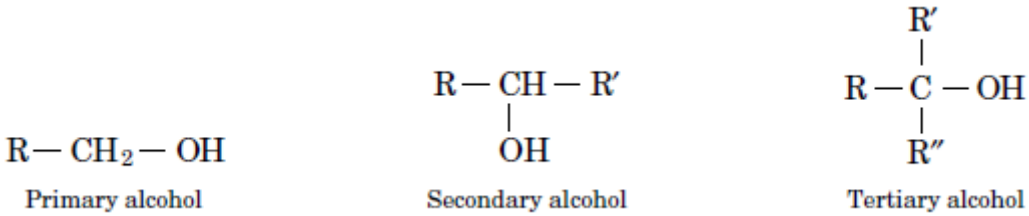
Introduction

Specific groups of atoms in an organic molecule can determine its physical and chemical properties. These groups are referred to as *functional groups*. Organic compounds which contain the functional group _OH, the hydroxyl group, are called *alcohols*.

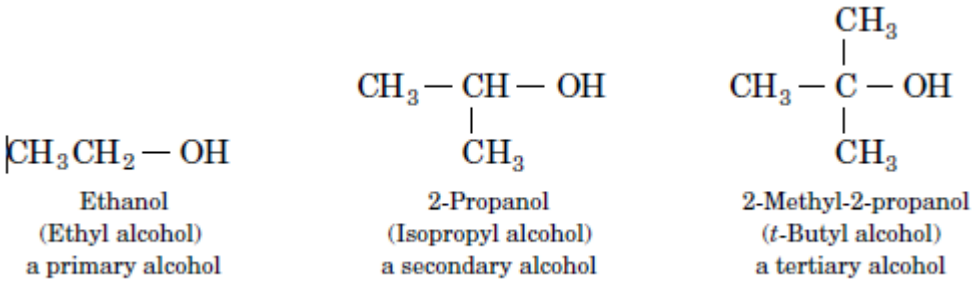
Alcohols are important commercially and include uses as solvents, drugs, and disinfectants. The most widely used alcohols are methanol or methyl alcohol, CH_3OH, ethanol or ethyl alcohol, CH_3CH_2OH, and 2-propanol or isopropyl alcohol, $(CH_3)_2CHOH$.

Methyl alcohol is found in automotive products such as antifreeze and "dry gas." Ethyl alcohol is used as a solvent for drugs and chemicals, but is more popularly known for its effects as an alcoholic beverage. Isopropyl alcohol, also known as "rubbing alcohol," is an antiseptic.

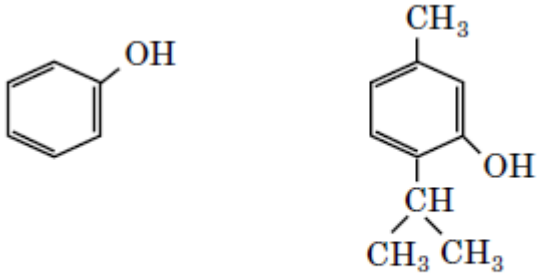
Alcohols may be classified as either primary, secondary, or tertiary:



Note that the classification depends on the number of carbon-containing groups, R (alkyl or aromatic), attached to the carbon bearing the hydroxyl group. Examples of each type are as follows:



Phenols bear a close resemblance to alcohols structurally since the hydroxyl group is present. However, since the _OH group is bonded directly to a carbon that is part of an aromatic ring, the chemistry is quite different from that of alcohols. Phenols are more acidic than alcohols; concentrated solutions of the compound phenol are quite toxic and can cause severe skin burns. Phenol derivatives are found in medicines; for example, thymol is used to kill fungi and hookworms.

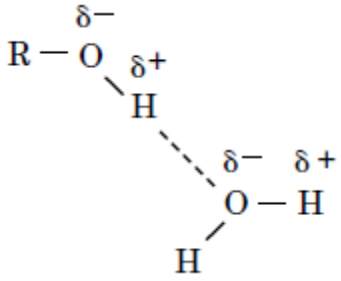


Phenol

Thymol
(2-isopropyl-5-methylphenol)

Physical Properties

Since the hydroxyl group is present in alcohols and phenols, these compounds are polar. The polarity of the hydroxyl group, coupled with its ability to form hydrogen bonds, enables many alcohols and phenols to mix with water. Since these compounds also contain nonpolar portions, they show additional solubility in many organic solvents, such as dichloromethane and diethyl ether.



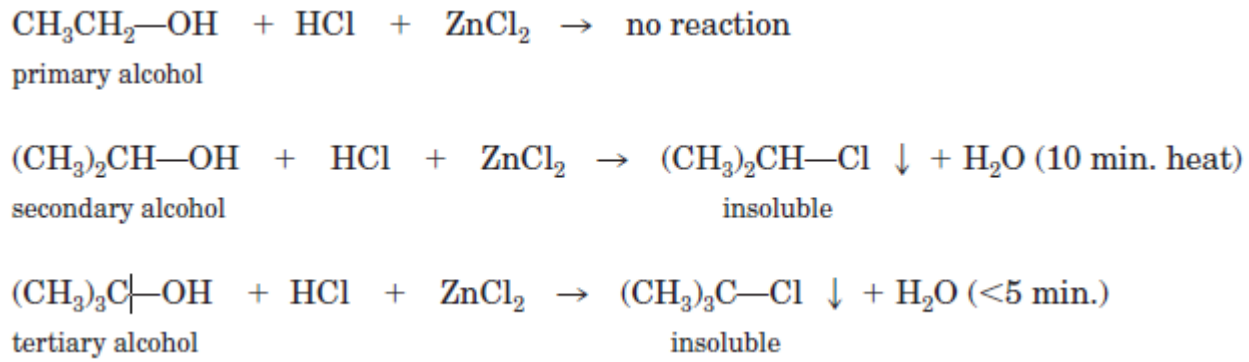
Hydrogen bonding of the hydroxyl group with water.

Chemical Properties

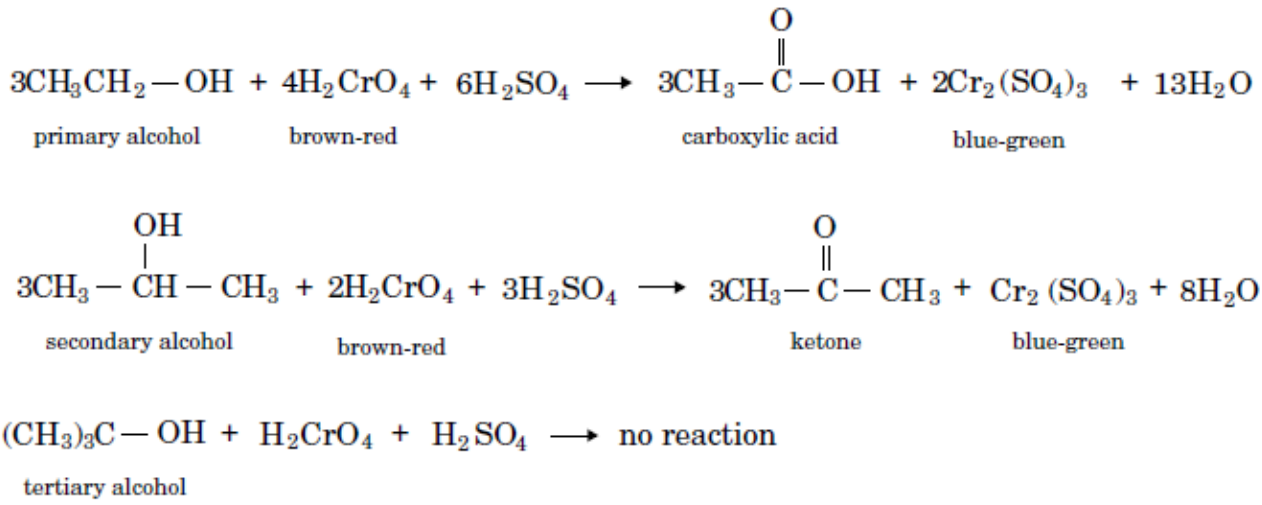
The chemical behavior of the different classes of alcohols and of phenols can be used as a means of identification. Quick, simple tests that can be carried out in test tubes will be performed.

1. *Lucas test*. This test is used to distinguish between water-soluble primary, secondary, and tertiary alcohols. Lucas reagent is a mixture of zinc chloride, ZnCl2, in concentrated HCl. Upon addition of this reagent, a tertiary alcohol reacts rapidly and immediately gives an insoluble white layer. A secondary alcohol reacts slowly and, after heating slightly, gives the white layer within 10 min. A primary alcohol does not react.

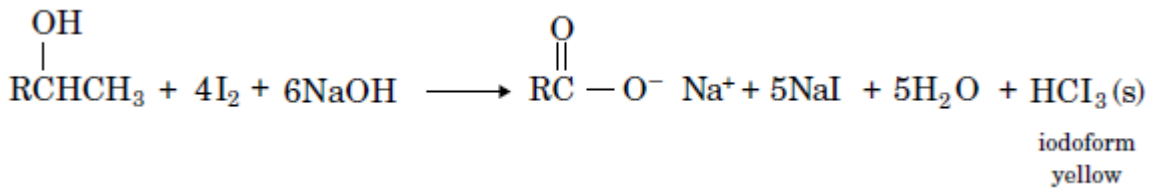
Any formation of a heterogeneous phase or appearance of an emulsion is a positive test.



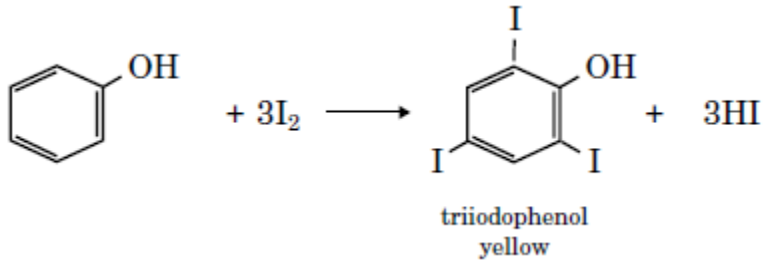
2. *Chromic acid test*. This test is able to distinguish primary and secondary alcohols from tertiary alcohols. Using acidified dichromate solution, primary alcohols are oxidized to carboxylic acids; secondary alcohols are oxidized to ketones; tertiary alcohols are not oxidized. (Note that in those alcohols which are oxidized, the carbon that has the hydroxyl group *loses a hydrogen*.) In the oxidation, the brown-red color of the chromic acid changes to a blue-green solution. Phenols are oxidized to nondescript brown tarry masses.



3. *Iodoform test*. This test is more specific than the previous two tests. Only ethanol (ethyl alcohol) and alcohols with the part structure CH3CH(OH) react. These alcohols react with iodine in aqueous sodium hydroxide to give the yellow precipitate iodoform.

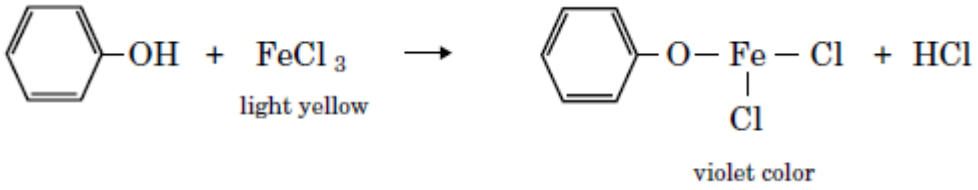


Phenols also react under these conditions. With phenol, the yellow precipitate triiodophenol forms.



4. *Acidity of phenol*. Phenol is also called carbolic acid. Phenol is an acid and will react with base; thus phenols readily dissolve in base solutions. In contrast, alcohols are not acidic.

5. *Iron(III)chloride test*. Addition of aqueous iron(III) chloride to a phenol gives a colored solution. Depending on the structure of the phenol, the color can vary from green to purple.



Materials

Table 3.1: Chemicals and supplies

Chemicals	Supplies
1-butanol	10 test tubes
2-butanol	pH paper
2-methyl-2-propanol	Big beaker (serve as water bath 60°C)
Phenol	Hot plate
Dioxane	Glass rod
6M NaOH	
I_2/KI solution	
Lucas reagent	
Acetone	
1 M Chromic acid, H_2CrO_4	
1M Iron (III) chloride, $FeCl_3$	
Unknown	

Note: The TA should prepare the necessary solutions before lab starts

Procedure

Part I: Physical Properties of Alcohols and phenols

1. Bring 4 separated test tubes and label them: 1-butanol, 2-butanol, 2-methyl-2-propanol, and unknown respectively then add 10 drops of each sample.

2. Add 3 mL of distilled water to each test tube.

3. Into a separate test tube, place 2 mL of a prepared water solution of phenol. Record your observations as soluble or insoluble sample in table 3.1 of the Report Sheet.

4. Test the pH of each of the aqueous solutions. Do the test by first dipping a clean glass rod into the solutions and then transferring a drop of liquid to pH paper and read the value of the pH by comparing the color to the chart on the dispenser. Record the results in table 3.1.

Part II: Chemical Properties of Alcohols and phenols

A. Iodoform Test

1. Label 5 clean, dry test tubes as 1-butanol, 2-butanol, 2-methyl-2-propanol, phenol, and unknown respectively then add 5 drops of each sample to be tested.

2. Add to each test tube 2 mL of water. If the compound is not soluble, add dioxane (dropwise) until the solution is homogeneous.

3. Add to each test tube (dropwise) 2 mL of 6 M NaOH; tap the test tube with your finger to mix.

4. Warmed the mixture in each test tube in a 60°C water bath for 5 minutes, and then add dropwise (with shaking) I_2/KI solution until the solution becomes brown (approx. 25 drops).

   Note: while it is in the water bath and if the color is fade, add more drops of I_2/KI solution until the dark color persists for 2 min.

5. Add 6 M NaOH (dropwise) until the solution becomes colorless. Keep the test tubes in the warm water bath for 5 min.

6. Remove the test tubes from the water bath, let it cool, and look for a light yellow precipitate. Record your observations in table 3.1.

   Note: If the formation of the yellow precipitate tends to be slow. Put these test tubes a side and make your observations at the end of the lab.

B. Lucas test

A. Place 5 drops of each sample into a clean, dry test tubes and labeled them as you did before.

B. Add 1 mL of Lucas reagent; mix well by stoppering each test tube with a cork, tapping the test tube sharply with your finger for a few seconds to mix; remove the cork after mixing and allow each test tube to stand for 6 min. Look carefully for any cloudiness that may develop during this time period.

   Note: If there is no cloudiness after 10 min., warm the test tubes that are clear for 15 min. in a 60°C water bath. Record your observations in table 3.1.

C. Chromic acid test

CAUTION! Chromic acid is toxic and corrosive. Handle with care and promptly wash any spill. Use gloves with this reagent

1. Repeat step 1 in part II (B).

2. To each test tube add 10 drops of acetone and 2 drops of 1M chromic acid. Place the test tubes in a 60°C water bath for 5 min. observe the color of each solution and Record your observations in table 3.1.

   Note: you will get a positive test when the brown-red will be lost and the blue-green color is formed.

D. Iron(III) chloride test

1. Repeat step 1 in part II (B).
2. Add 2 drops of 1M iron(III) chloride solution to each test tube and observe any color changes in each solution. Record your observations in table 3.1.

   Note: a purple color indicates the presence of a phenol

Report Sheet 1

Date:

Grade

Group Names:

Table 3.2: Recording Observation

Test	1-butanol	2-butanol	2-methy-2-propanol	Phenol	Unknown
Solubility in H_2O					
pH test					
Iodoform					
Lucas					
H_2CrO_4 acid					
$FeCl_3$					

Identity of unknown:

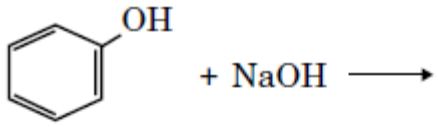
Unknown no. _____. The unknown compound is _____.

Report Sheet 2

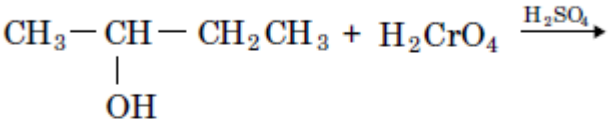
Answer the following questions:

1. Write the structure of the major organic product expected from each of the following reactions. If no reaction is expected write "No Reaction."

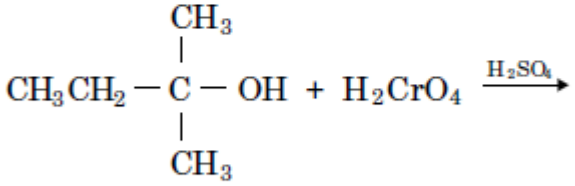
a.



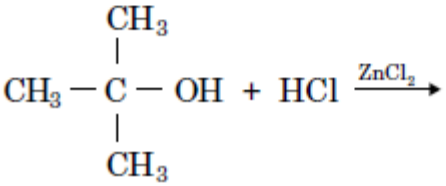
b.



c.



d.



2. A student had two unknown liquid alcohols. Unknown A gave a blue-green color with chromic acid and formed a precipitate after heating for 10 min. with Lucas reagent. Unknown B showed no color change with chromic acid but formed an immediate precipitate with Lucas reagent. To which alcohol classes do alcohols A and B belong?

3. What simple test can be used to distinguish between an alcohol and a phenol?

Experiment 4

Identification of Aldehydes and Ketones

Objectives

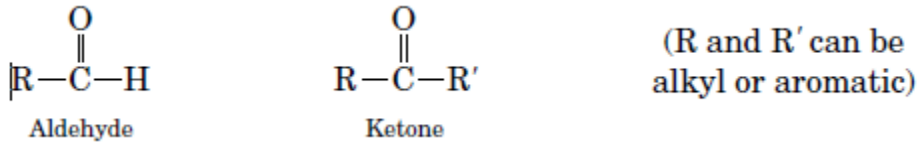
1. To learn the chemical and physical characteristics of aldehydes and ketones.
2. To use these chemical characteristics in simple tests to distinguish between examples of aldehydes and ketones.

Introduction

Aldehydes and ketones are representative of compounds which possess the carbonyl group:

The carbonyl group

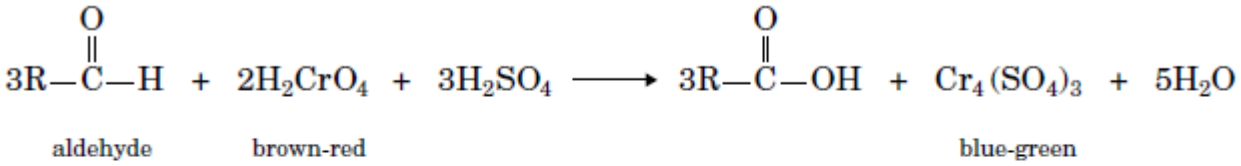
Aldehydes have at least one hydrogen attached to the carbonyl carbon; in ketones, no hydrogen is directly attached to the carbonyl carbon, only carbon containing R-groups:

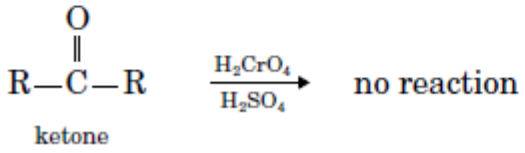


Aldehydes and ketones of low molecular weight have commercial importance. Many others occur naturally.

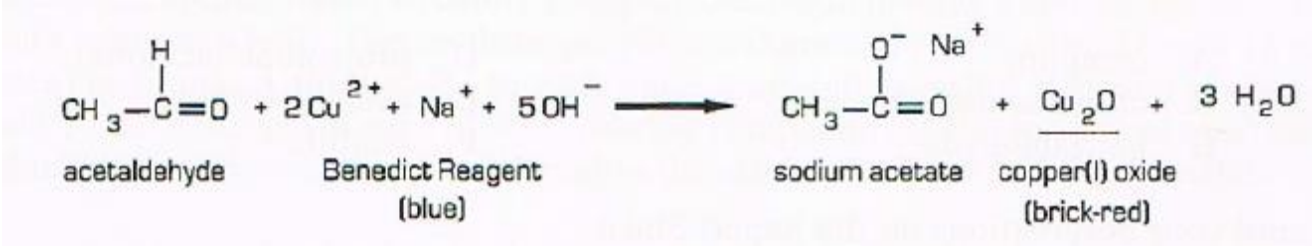
Classification Tests

1. *Chromic acid test*. Aldehydes are oxidized to carboxylic acids by chromic acid; ketones are not oxidized. A positive test results in the formation of a blue-green solution from the brown-red color of chromic acid.

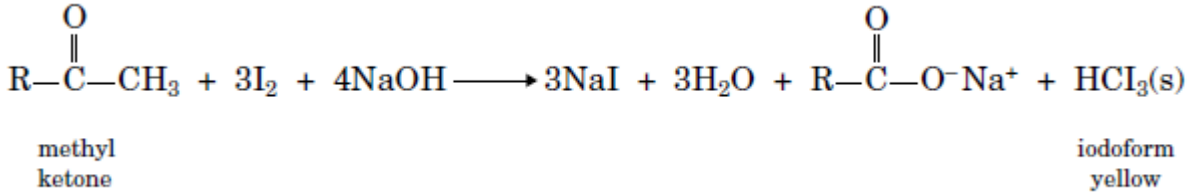




2. *Benedicts' test*. Is a very sensitive test in which the oxidation of aldehyde is accompanied by simultaneous reduction of copper(II) ion Cu^{2+} to a highly colored precipitate of copper(I) oxide, Cu_2O. The appearance of the color product signals the oxidation of an aldehyde to the carboxylic acid. Most often the copper(I) oxide, Cu_2O forms as a dark red precipitate but it can also produce a yellow or orange-yellow color too. Any red-orange-yellow color indicate the presence of an aldehyde.
   The Benedict reagent is basic (NaOH) solution of copper sulfate, $CuSO_4$. Because the test solution is basic, the sodium salt of the carboxylic acid is produced:



3. *Iodoform test*. Methyl ketones give the yellow precipitate iodoform when reacted with iodine in aqueous sodium hydroxide.



Materials

4.1: Chemicals and supplies

Chemicals	Supplies
Propanal	6 test tubes with lid or cork or Parafilm
2-methyl propanal	400 mL beaker serve as boiling water bath
n-butyl aldehyde	rack
Glucose	10 mL graduated cylinder
Acetone	
cyclohexanone	
Ethanol	
2-propanol	
4-hyptanone	
*1 M Chromic acid	
**Benedict reagent	

****Benedict reagent: Solution 1: dissolve 1.73 g hydrated $CuSO_4$ crystals in 20 mL distilled water. Solution 2: dissolve 10 g anhydrous Na_2CO_3 in 70 mL distilled water. Add solution 1 to solution 2 and complete the volume to 100 ml volumetric flask.**

****TA should prepare the necessary solutions***

Procedure

Part I: Physical properties of Aldehydes and Ketones

A. Solubility of Aldehydes and Ketones

1. Label 6 test tubes as propanal, 2-methyl propanal, n-butyl aldehyde, glucose, acetone, and 4-hyptanone respectively then add 10 drops of each sample to be tested.

   Note: glucose is solid, you should add small amount of glucose particles in the test tube equal to the size of the head of a match.

2. Add 1 mL of distilled water to each test tube and seal each test tube with a cork or Parafilm and shake each mixture thoroughly, then set it aside in a test tube rack.

3. Wait for 5 minutes and record your observation in table 4.2 in the report sheet

   Note: if the sample is insoluble in water you should observe either a cloudy mixture or two separated layers.

Part II: Chemical Properties of Aldehydes and Ketones

A. Chromic Acid Test

1. Label 5 clean test tubes as propanal, 2-propanol, ethanol, acetone (Control), and 4-hyptanone respectively then add 4 drops of each sample to be tested.

2. Add 1 mL of acetone to each test tube (except the acetone test tube

   CAUTION! Chromic acid is toxic and corrosive. Handle with care and promptly wash any spill. Use gloves with this reagent

3. Add one drop of chromic acid to the acetone test tube only (the color of the mixture should remain orange).

   Note: if the color change, you should repeat step 3 in another clean test tube

4. Seal each test tube with a cork or Parafilm and shake each mixture thoroughly.

5. Add one drop of chromic acid to each test tube and shake the mixture again and observe any change in color within 10 seconds and record your Observation in table 4.3 in the report sheet.

6. Set the test tubes aside in a test tube rack for 5 minutes for more evident observation.

B. Benedict Test

1. Put 200 mL water in a 400 mL beaker and heat it till boiling.

2. Label 5 clean test tubes as distilled water (Control), propanal, ethanol, glucose, and acetone respectively

3. Add 2 mL of Benedict reagent to each test tube.

4. Add 1 mL of the substance to each of the labeled samples to be tested.

   Note: glucose is solid, you should add small amount of glucose particles in the test tube equal to the size of the head of a match.

5. Seal each test tube with a clean cork or Parafilm and mix thoroughly.

6. Place each labeled in a boiling water bath for 10 minutes.

7. Remove and place the test tubes in a test tube rack, as the test tubes cool check for a dark red, orange, brown, yellow precipitate and record the observation in table 4.3 in the report sheet

Report Sheet 1

Grade

Group Names:

Date:

Table 4.2: Test the Solubility of the substances in water

2-heptanone	Propanal	2-methylpropanal	Heptanal	Glucose	Acetone

Table 4.3: Chemical Properties of Aldehydes and Ketones

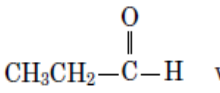
Test	Acetone	Ethanol	2-propanol	Propanal	2-heptanone	Glucose
Chromic acid						---
Benedict Reagent			---		---	

Answer the following question:

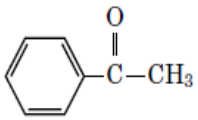
1. What happens to an aldehyde in its reaction with chromic acid?

2. What is the result of oxidation of 2-propanol?

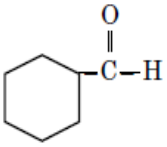
3. What kind of results do you see when the following compounds are mixed together with the given test solution?



with chromic acid



with I_2-KI reagent



Benedict reagent

Experiment 5

Synthesis of O-Chlorobenzoic acid

Objectives

1. Carry out a diazotization reaction on anthranilic acid to prepare the corresponding diazonium salt.
2. Carry out a Sandmeyer reaction on the diazonium salt to prepare an aryl halide.

Introduction

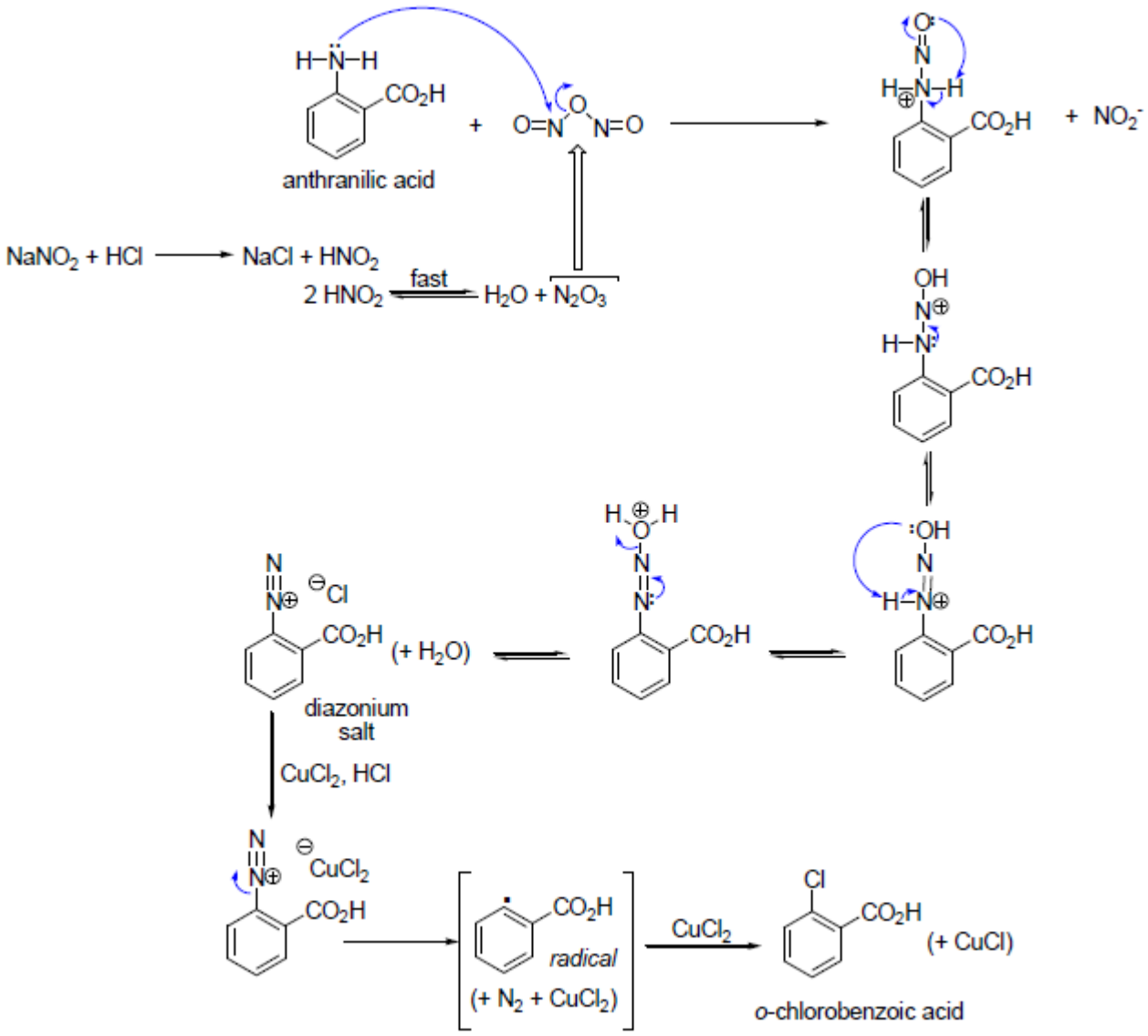
Primary arylamines react with nitrous acid, HNO_2, to yield stable arenediazonium salts, $Ar\text{-}N^{+}\equiv N^{-}\text{-}X$,a process called *diazotization* reaction. Alkylamines also react with nitrous acid, but the corresponding alkanediazonium products are so reactive they can't be isolated. Instead they lose nitrogen instantly to yield carbocations. The analogous loss of N_2 from an arenediazonium ion to yield an aryl cation is disfavored by the instability of the cation.

Arene diazonioum salts are useful because the diazonio group (N_2) can be replaced by a nucleophile in a substitution reaction. Many different nucleophiles – halide, hydride, cyanide, and hydroxide among others – react with arenediazonium salts, yielding many different kinds of substituted benzenes. The overall sequence of (1) nitration, (2) reduction, (3) diazotization, (4) nucleophilic substitution is perhaps the single most versatile method of aromatic substitution.

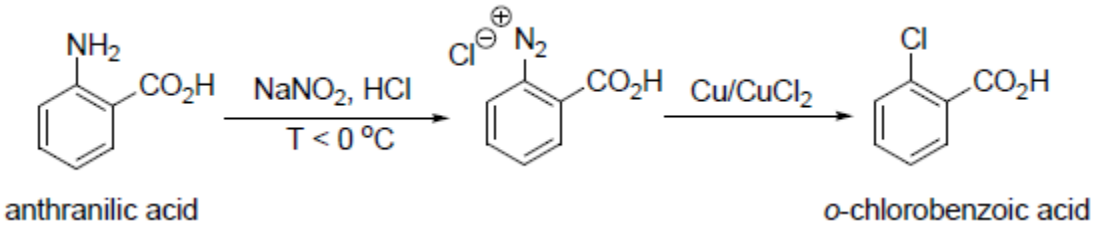
Aryl chlorides and bromides are prepared by reaction of an arenediazonium salt with the corresponding copper(I) halide, CuX, a process called the **Sandmeyer reaction**.

Mechanism

Mechanistically, the diazonio replacement reaction occur through radical rather than polar pathways. In the presence of a copper(I) compound, for instance, it's thought that the arenediazonium ion is first converted to an aryl radical plus copper(II), followed by subsequent reaction to give product plus regenerated copper(I) catalyst.


anthranilic acid
NaNO2 + HCl
NaCl + HNO2
2 HNO2
fast
H2O + N2O3
+ NO2-
diazonium salt
(+ H2O)
CuCl2, HCl
CuCl2
radical
(+ N2 + CuCl2)
(+ CuCl)
o-chlorobenzoic acid

Reaction


NaNO2, HCl
T < 0 °C
Cu/CuCl2
anthranilic acid
o-chlorobenzoic acid

Materials

Table 5.1: Chemicals and Supplies

Chemical	Supplies
$CuCl_2.H_2O$	125 mL Erlenmeyer Flask
Copper metal (small pieces)	250 mL Erlenmeyer Flask
N-Phenyl Anthranilic acid	Salt-ice water bath
Conc HCl	Hot plate
Cold conc HNO_2	600 mL beaker
$NaNO_2$	Glass rod and thermometer
	Büchner funnel, filter flask, filter paper (Fig 5.1)
	50 mL graduated cylinder

Procedure

Part I: Preparing cold solution of $CuCl_2$

1. In a 125 mL Erlenmeyer flask weigh 4.7 g $CuCl_2.2H_2O$.

2. Add 20 mL of water to step 1 and stir until complete dissolution.

3. Add 15 mL of concentrated HCl to step 1 and 3.5 g copper metal and heat the mixture until it boils gently.

4. Keep boiling for about 15 min time after which a decoloration should be observed. Meanwhile, carry out the following process:

Part II: Diazotization process

1. Measure 10 mL concentrated HCl and transfer it to a 250 mL Erlenmeyer flask and add 50 mL water.

2. Add 5.5 g N-Phenyl Anthranilic acid acid to step 1 and Dissolve it by heating slightly and then cool the solution in an ice-salt bath and leave it there.

3. While monitoring the temperature, in a beaker prepare a solution of $NaNO_2$ by dissolving 2.8 g of $NaNO_2$ in 10 mL water

4. Add dropwise of solution in step 3 to the anthranilic acid solution in the 250 mL Erlenmeyer flask.

   Note: The temperature of the mixture should not exceed 0.0°C.

5. Once the addition is over, keep the Erlenmeyer flask in the ice-salt bath.

Part III: Sandmeyer reaction

1. In a 600 mL beaker, add the $CuCl_2$ solution from part I and cool it down quickly below 0 °C.
2. Add the diazonium salt from part II gradually while stirring vigorously with a glass rod.

   Note: A large amount of foam is produced due to the release of nitrogen gas.
3. Keep stirring for 30 min. Then, filter over a Büchner funnel, figure 5.1, and wash the precipitate, first with cold ~8 *M* HNO_2, then with cold water until the filtrate gets colorless.
4. Dry the precipitate over vacuum.
5. Weigh the mass of crude product (o-chlorobenzoic acid) obtained.

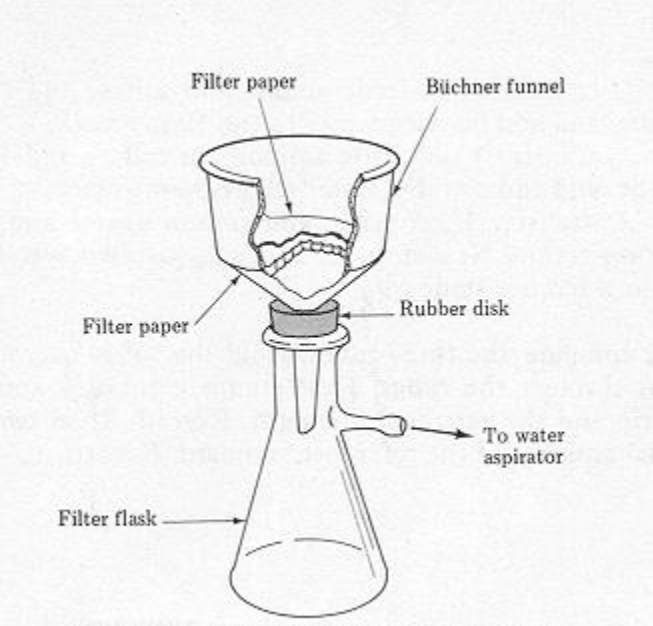


Figure 5.1: Apparatus for suction filtration

Report Sheet 1

Grade

Group Names:

Date:

Mass of pure o-chlorobenzoic acid expected: 6.28 g

Mass of crude o-chlorobenzoic acid obtained: _____ g

Percent yield in crude product = $\frac{Mass\ of\ crude\ product\ obtained}{Mass\ of\ pure\ product\ expected}$ × 100 =

= _____ × 100

= %

1. What is the purpose of washing the anthranilic acid with HNO_2 solution?

2. Give another example of nucleophile that could react with the diazonium salt. Write the equation.

Experiment 6

Esterification Reaction: Preparation of Aspirin

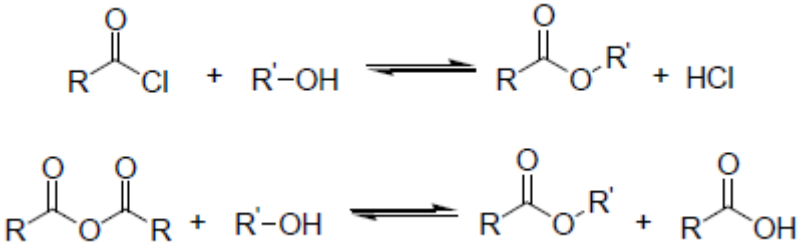
Objectives

1. Synthesize aspirin from its corresponding acid anhydride and alcohol
2. Compare two different synthetic routes for the preparation of esters

Introduction

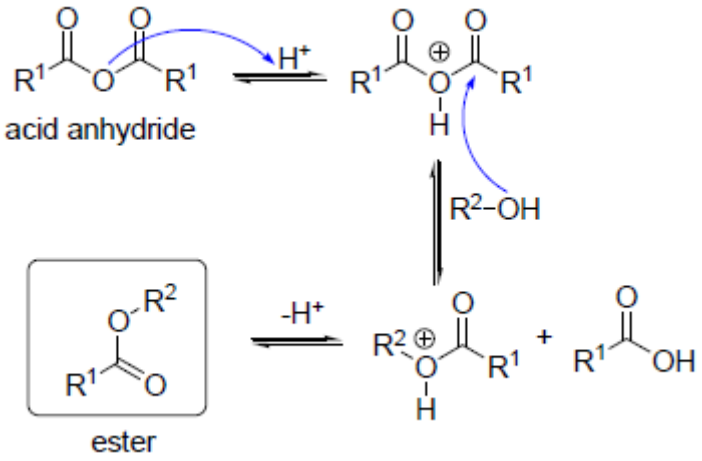
The classic synthesis of esters is the Fischer-Speier esterification, employed in experiment 1. However, several other methods are available, one being often favored other another depending on the problems needing to be tackled. The method used in this experiment is the alcoholysis of an acid anhydride.

Alcohols react with acyl chlorides or acid anhydrides to give esters:

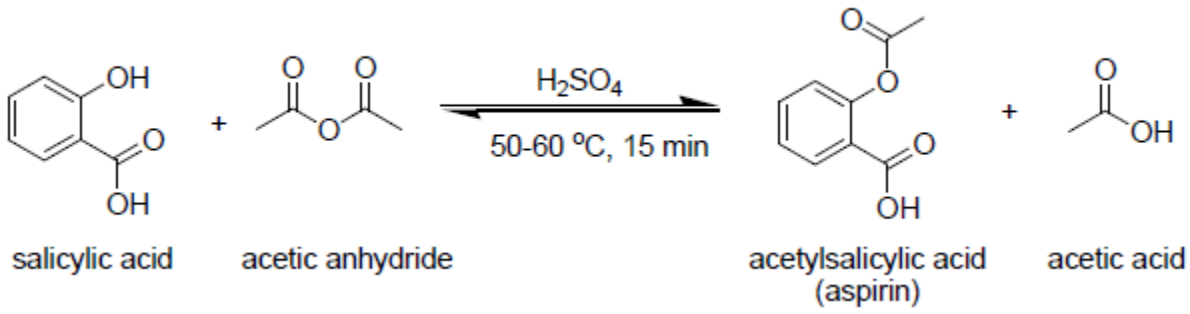


These reactions are irreversible, thus simplifying workup. Since acyl chlorides and acid anhydrides react also with water, anhydrous conditions are preferred. The analogous acylation of amines that produces amides is less sensitive towards water because amines are stronger nucleophiles and react more rapidly.

General Mechanism



Reaction



Materials

Table 6.1: Chemicals and supplies

Chemicals	Supplies
salicylic acid (2 g)	50 mL beaker
acetic anhydride (3 mL)	thermometer
conc. sulfuric acid (1 drop)	glass rod
	Büchner funnel, filter paper
	melting point apparatus
	Water bath (bain-marie) 50-60°C
	10 mL graduated cylinder

Procedure

1. Weight 2.0 g of salicylic acid using a 50 mL beaker.
2. Measure 3 mL of acetic anhydride using the graduated cylinder and add it to step 1.
3. Add 1 drop of concentrated sulfuric acid to the mixture in the beaker with stirring.
4. Heat the mixture using the water bath 50-60°C for 15 minutes with continuous stirring using a glass rod.
5. Add 35 mL of distilled water, swirl the mixture and filter it using vacuum filtration (refer to figure 5.1)
6. Weigh the mass of crude product (aspirin) obtained

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Mass of pure aspirin expected: 2.61 g

Mass of crude aspirin obtained: _____ g

Percent yield in crude product = $\frac{Mass\ of\ crude\ product\ obtained}{Mass\ of\ pure\ product\ expected}$ × 100 =

= _____ × 100

= %

Give an alternative method of synthesis of aspirin, using salicylic acid as a stating material. Give the mechanism.

Experiment 7

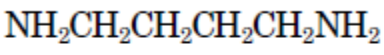
Properties of Amine and Amides

Objectives

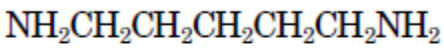
1. To show some physical and chemical properties of amines and amides.
2. To demonstrate the hydrolysis of amides.

Introduction

Amines and amides are two classes of organic compounds which contain nitrogen. Amines behave as organic bases and may be considered as derivatives of ammonia. Amides are compounds which have a carbonyl group connected to a nitrogen atom and are neutral. In this experiment, you will learn about the physical and chemical properties of some members of the amine and amide families.
If the hydrogens of ammonia are replaced by alkyl or aryl groups, amines result.
Depending on the number of carbon atoms bonded directly to nitrogen, amines are classified as either primary (one carbon atom), secondary (two carbon atoms), or tertiary (three carbon atoms).
There are a number of similarities between ammonia and amines that carry beyond the structure. Consider odor. The smell of amines resembles that of ammonia but is not as sharp. However, amines can be quite pungent. Anyone handling or working with raw fish knows how strong the amine odor can be, since raw fish contains low-molecular-weight amines such as dimethylamine and trimethylamine. Other amines associated with decaying flesh have names suggestive of their odors: putrescine and cadaverine.



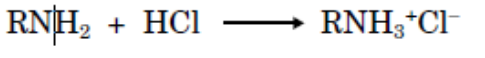
Putrescine
(1,4-Diaminobutane)



Cadaverine
(1,5-Diaminopentane)

The solubility of low-molecular-weight amines in water is high. In general, if the total number of carbons attached to nitrogen is four or less, the amine is water soluble; amines with a carbon content greater than four are water insoluble. However, all amines are soluble in organic solvents such as diethyl ether or dichloromethane.

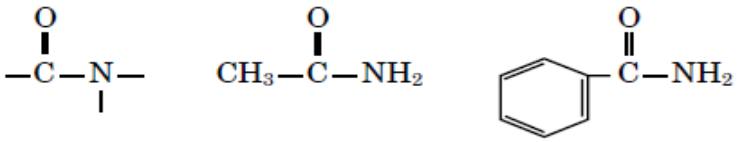
Since amines are organic bases, water solutions show weakly basic properties. If the basicity of aliphatic amines and aromatic amines are compared to ammonia, aliphatic amines are stronger than ammonia, while aromatic amines are weaker. Amines characteristically react with acids to form ammonium salts; the non-bonded electron pair on nitrogen bonds the hydrogen ion.



Amine Ammonium Salt

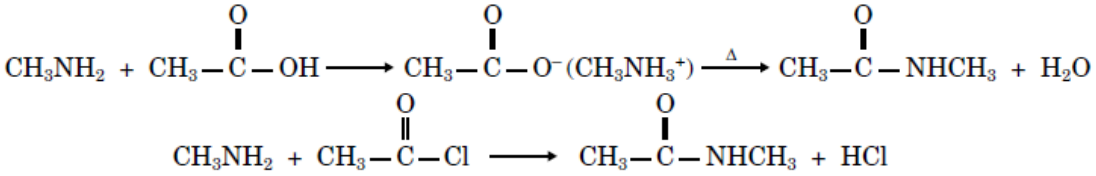
If an amine is insoluble, reaction with an acid produces a water-soluble salt. Since ammonium salts are water soluble, many drugs containing amines are prepared as ammonium salts. After working with fish in the kitchen, a convenient way to rid one's hands of fish odor is to rub a freshly cut lemon over the hands. The citric acid found in the lemon reacts with the amines found on the fish; a salt forms which can be easily rinsed away with water.

Amides are carboxylic acid derivatives. The amide group is recognized by the nitrogen connected to the carbonyl group. Amides are neutral compounds; the electrons are delocalized into the carbonyl (resonance) and thus, are not available to bond to a hydrogen ion.

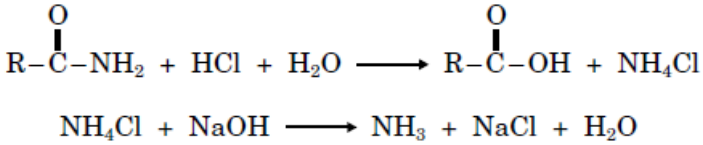


Amide group Acetamide Benzamide

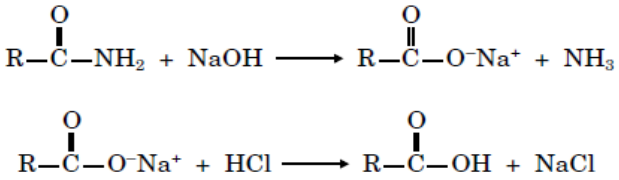
Under suitable conditions, amide formation can take place between an amine and a carboxylic acid, an acyl halide, or an acid anhydride. Along with ammonia, primary and secondary amines yield amides with carboxylic acids or derivatives.



Hydrolysis of amides can take place in either acid or base. Primary amides hydrolyze in acid to ammonium salts and carboxylic acids. Neutralization of the acid and ammonium salts releases ammonia which can be detected by odor or by litmus.



Secondary and tertiary amides would release the corresponding alkyl ammonium salts which, when neutralized, would yield the amine.
In base, primary amides hydrolyze to carboxylic acid salts and ammonia. The presence of ammonia (or amine from corresponding amides) can be detected similarly by odor or litmus. The carboxylic acid would be generated by neutralization with acid.



Materials

Table 7.1: Chemicals and supplies

Chemicals	supplies
6 M ammonia	5 small test tubes
Triethylamine	Watch glass
Aniline	1 large test tubes
N,N-dimethylaniline	Boiling water bath
N,N-Dimethyl Acetamide	pH-paper
6 M HCl	Ice-water bath
Diethyl ether	10 mL graduated cylinder
Conc. HCl	
6 M H_2SO_4	
6 M NaOH	

Note: TA must prepare the necessary solutions before lab starts.

Procedure

CAUTION!

Amines are toxic chemicals. Avoid excessive inhaling of the vapors and use gloves to avoid direct skin contact. Anilines are more toxic than aliphatic amines and are readily absorbed through the skin. Wash any amine or aniline spill with large quantities of water. Diethyl ether (ether) is extremely flammable. Be certain there are **NO** open flames in the immediate area.

Part I: Properties of Amines

1. Label 5 clean, dry test tubes as NH_3, Triethylamine, Aniline, N,N-Dimethylaniline, and N,N-Dimethyl Acetamide respectively then add 5 drops (or about 0.1 g if solid) of each sample to be tested.

2. Add 2 mL of distilled water to each of the labeled test tubes. Mix thoroughly by sharply tapping the test tube with your finger. Record your observation in table 7.2 in the report sheet whether the amines are soluble or insoluble.

3. Take a glass rod and carefully dip one end of the glass rod into a solution and touch a piece of pH paper. Between each test, be sure to clean and dry the glass rod. Record the pH of each solution.

4. Carefully add 2 mL of 6 M HCl to each test tube. Mix thoroughly by sharply tapping the test tube with your finger. Record your observation in table 7.2 in the report Sheet whether the amines are soluble or insoluble.

5. Repeat step 1 and Add 2 mL of diethyl ether (ether) to each test tube. Stopper with a cork and mix thoroughly by shaking. Record the observed solubility in table 7.2.

6. Carefully place on a watch glass, side-by-side, without touching, a drop of triethylamine and a drop of concentrated HCl. Record your observations in the report sheet.

Part II: Hydrolysis of acetamide

1. In a large clean, dry test tubes put about 0.5 g of N,N-Dimethyl Acetamide.

2. Measure 5 mL of 6 M H_2SO_4 using a graduated cylinder and add it to step 1 to dissolve the acetamide.

3. Heat the solution in a boiling water bath for 5 min.

4. Hold a small strip of moist pH paper over the mouth of the test tube; observe any changes in color; record the pH reading in table 7.3.

5. Remove the test tube from the water bath, holding it in a test tube holder. Record your feeling if there is any odor in table 7.3.

6. Place the test tube in an ice water bath until cool to the touch.

7. Carefully add, dropwise with shaking, 6 M NaOH to the cool solution until basic.

   Note: You may need more than 7 mL of base.

8. Hold a piece of moist pH paper over the mouth. Record the pH reading in table 7.3 in addition to record your feeling if there is any odor.

Report Sheet 1

Date:

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Table 7.2: Physical properties of Amines and Amides

	Odor		Solubility			pH
	Original solution	With HCl	H_2O	Ether	HCl	H_2O
NH_3						
Triethylamine						
Aniline						
N,N-Dimethylaniline						
Acetamide						

Triethylamine and concentrated hydrochloric acid observation:

Table 7.3: Hydrolysis of Acetamide

	PH	Odor	Any color change of solution
heating the solution			
After heating	-------		-------
Adding NaOH			

Report Sheet 2

Answer the following questions:

1. From table 7.2, Compare between the observations of the solubility of adding HCl to each sample with the observation of adding water to them?

2. Write the structure of the salt that forms when diethylamine, (CH3CH2)2NH, is mixed with hydrochloric acid.

3. Write the chemical equation for the reaction of triethylamine with concentrated hydrochloric acid.

4. Write the equations that account for what happens in the hydrolysis of the acetamide solution in (a) acid and in (b) base.

a.

b.

Experiment 8

Carbohydrates

Objectives

1. To become familiar with the reducing or non-reducing nature of carbohydrates.
2. To experience the enzyme-catalyzed and acid-catalyzed hydrolysis of acetal groups.

Introduction

Carbohydrates are polyhydroxy aldehydes, ketones, or compounds that yield polyhydroxy aldehydes or ketones upon hydrolysis. Rice, potatoes, bread, corn, candy, and fruits are rich in carbohydrates. A carbohydrate can be classified as a monosaccharide (glucose or fructose); a disaccharide (sucrose or lactose), which consists of two joined monosaccharide; or a polysaccharide (starch or cellulose), which consists of thousands of monosaccharide units linked together. Monosaccharide exist mostly as cyclic structures containing hemiacetal (or hemiketal) groups. These structures in solutions are in equilibrium with the corresponding open chain structures bearing aldehyde or ketone groups. Glucose, blood sugar, is an example of a polyhydroxy aldehyde. Fig 8.1:

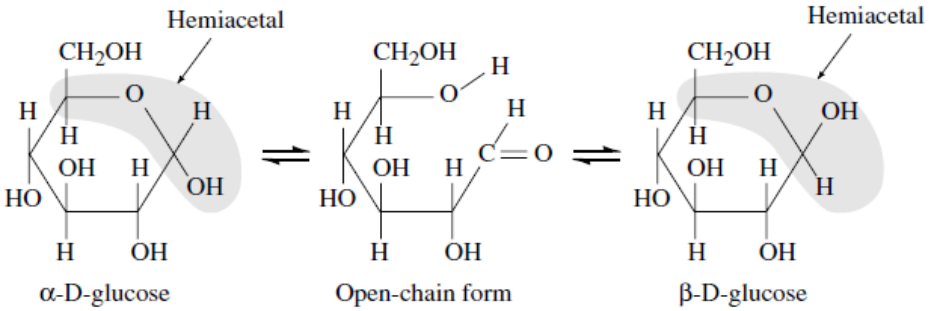


Figure 8.1: The structure of D-

Disaccharides and polysaccharides exist as cyclic structures containing functional groups such as hydroxyl groups, acetal (or ketal), and hemiacetal (or hemiketal). Most of the di-, oligo-, or polysaccharides have two distinct ends. The one end which has a hemiacetal (or hemiketal) on its terminal is called the reducing end, and the one which does not contain a hemiacetal (or hemiketal) terminal is the nonreducing end. The name "reducing" is given because hemiacetals (and to a lesser extent hemiketals) can reduce an oxidizing agent such as Benedict's reagent. Fig 8.2 is an example:

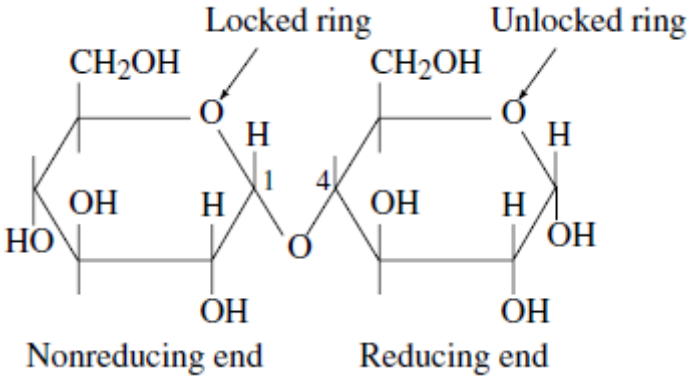


Figure 8.2: The structure of maltose, a disaccharide.

Not all disaccharides or polysaccharides contain a reducing end. An example is sucrose, which does not have a hemiacetal (or hemiketal) group on either of its ends (Fig. 8.3).

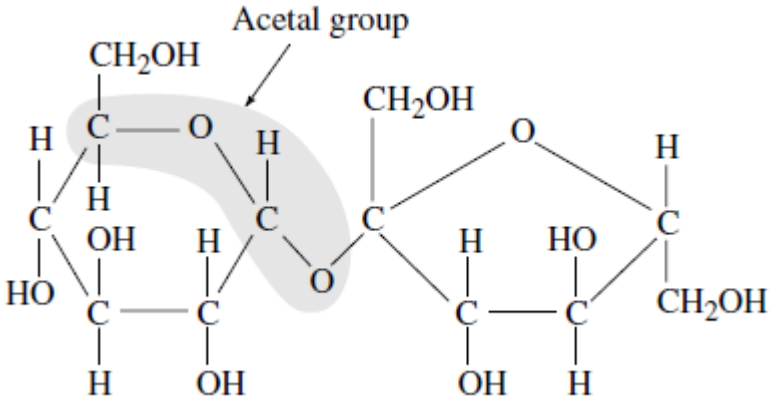


Figure 8.3: The structure of sucrose

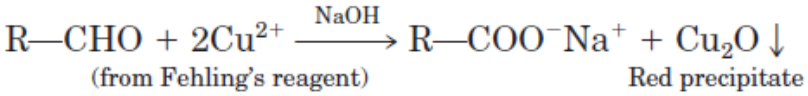
Polysaccharides, such as amylose or amylopectin, do have a hemiacetal group on one of their terminal ends, but practically they are nonreducing substances because there is only one reducing group for every 2,000–10,000 monosaccharidic units. In such a low concentration, the reducing group does not give a positive test with Benedict's or Fehling's reagent.

On the other hand, when a nonreducing disaccharide (sucrose) or a polysaccharide such as amylose is hydrolyzed the glycosidic linkages (acetal) are broken and reducing ends are created. Hydrolyzed sucrose (a mixture of D-glucose and D-fructose) will give a positive test with Benedict's or Fehling's reagent as well as hydrolyzed amylose (a mixture of glucose and glucose containing oligosaccharides). The hydrolysis of sucrose or amylose can be achieved by using a strong acid such as HCl or with the aid of biological catalysts (enzymes).

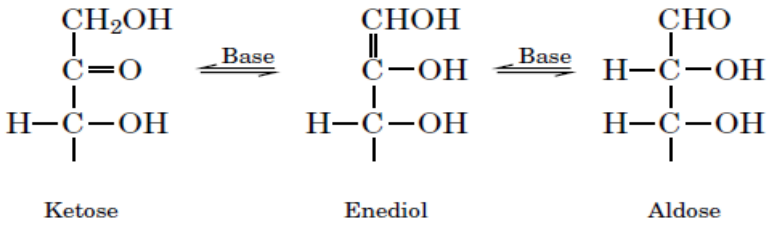
Starch can form an intense, brilliant, dark blue-, or violet-colored complex with iodine. The straight chain component of starch, the amylose, gives a blue color while the branched component, the amylopectin, yields a purple color. In the presence of iodine, the amylose forms helixes inside of which the iodine molecules assemble as long polyiodide chains. The helix-forming branches of amylopectin are much shorter than those of amylose. Therefore, the polyiodide chains are also much shorter in the amylopectin-iodine complex than in the amylose-iodine complex. The result is a different color (purple). When starch is hydrolyzed and broken down to small carbohydrate units, the iodine will not give a dark blue (or purple) color. The iodine test is used in this experiment to indicate the completion of the hydrolysis.

In this experiment, you will investigate some chemical properties of carbohydrates in terms of their functional groups.

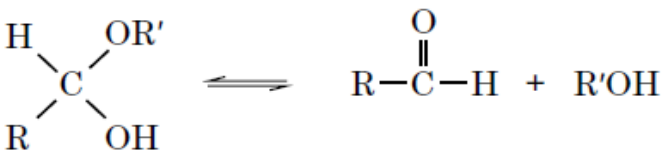
1. ***Reducing and non-reducing properties of carbohydrates***
   - **A. Aldoses (polyhydroxy aldehydes).** All aldoses are reducing sugars because they contain free aldehyde functional groups. The aldehydes are oxidized by mild oxidizing agents (e.g., Benedict's or Fehling's reagent) to the corresponding carboxylates. For example,



   - **B. Ketoses (polyhydroxy ketones).** All ketoses are reducing sugars because they have a ketone functional group next to an alcohol functional group. The reactivity of this specific ketone (also called α-hydroxyketone) is attributed to its ability to form an α-hydroxyaldehyde in basic media according to the following equilibrium equations:

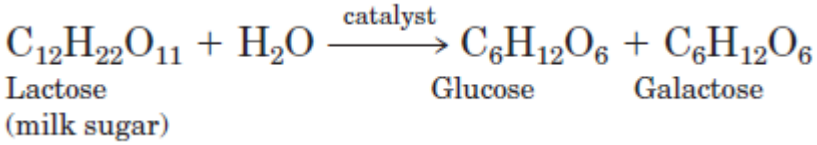


   - **C. Hemiacetal functional group (potential aldehydes).** Carbohydrates with hemiacetal functional groups can reduce mild oxidizing agents such as Benedict's reagent because hemiacetal can easily form aldehydes through the following equilibrium equation:



Sucrose is, on the other hand, a nonreducing sugar because it does not contain a hemiacetal functional group. Although starch has a hemiacetal functional group at one end of its molecule, it is, however, considered as a nonreducing sugar because the effect of the hemiacetal group in a very large starch molecule becomes insignificant to give a positive Benedict's test.

2. ***Hydrolysis of acetal groups.*** **Disaccharides and polysaccharides can be converted into monosaccharide by hydrolysis. The following is an example:**



Materials

Table 8.1: Chemicals and supplies

Chemicals	Supplies
Fehling's reagen	8 test tubes
2% Glucose	1 big test tube
2% Fructose	600 mL beaker serve as boiling water bath
2% Sucrose	Boiling chips in the bath
2% Lactose	10 mL graduated cylinder
2% Starch	Well plate
0.01 I_2-KI solution (Iodine solution)	droppers
3 M H_2SO_4	
3 M NaOH	
Saliva (Volunteer from each group)	

Note: TA must prepare the necessary solutions before lab starts.

Procedure

Part I: Reducing or Non-reducing Carbohydrates

1. In 600-mL beaker put about 200 mL of tap water with a few boiling chips and heat it till boiling and use it as water bath during the experiment.

2. Label 5 clean test tubes as Glucose, Fructose, Sucrose, Lactose, and Starch respectively then add 10 drops of each sample to be tested.

3. Add 2 mL (approximately 40 drops) of Fehling's solution to each test tube.

4. Place the test tubes in a boiling water bath prepared in step 1 for 5 min.

5. Record your results in table 8.2 in the report sheet. Which of those carbohydrates are reducing carbohydrates?

Part II: Hydrolysis of Carbohydrates

A. Hydrolysis of sucrose (acid versus base catalysis)

1. Label two test tubes as (no. 1 and no. 2) then and place in each 3 mL of 2% sucrose
2. To the first test tube (no. 1), add 3 mL of water and 3 drops of 3 M H_2SO_4 solution
3. To the second test tube (no. 2), add 3 mL of water and 3 drops of 3 M NaOH solution
4. Heat the test tubes in a boiling water bath prepared in part I-step 1 for about 5 min.
5. Cool both solutions to room temperature. To the contents of test tube no. 1, add about 10 drops of 3 M NaOH solution or until red litmus paper turns blue.
6. Test a few drops of each of the two solutions (test tube nos. 1 and 2) using the well plate with Fehling's reagent. Record the observation color of the solutions in table 8.3 in the report sheet.

B. Hydrolysis of starch (enzyme versus acid catalysis)

1. Label a new two clean test tubes as (no. 1 and no. 2) then and place in each 2 mL of 2% starch solution in each of two labeled test tubes.
2. To the first test tube (no. 1), add 2 mL of your own saliva.

   Note: Use a 10-mL graduated cylinder to collect your saliva.
3. To the second test tube (no. 2), add 2 mL of 3 M H_2SO_4 solution.
4. Place both test tubes in a water bath that has been previously heated to 45°C then allow the test tubes with their contents to stand in the warm water bath for 30 min.
5. Transfer a few drops of each solution into well plate cells and to each sample add 2 drops of iodine solution. Record the observation color of the solutions in table 8.3 in the report sheet.

C. Acid catalyzed hydrolysis of starch

1. Place 5 mL of starch solution in a large test tube and add 1 mL of 3 M H_2SO_4 solution. Mix it by gently shaking the test tube.

2. Heat the solution in a boiling water bath for about 5 min.

   Note: keep tracking the time for every 5 min heating to be able to fill table 8.3.

3. Using a clean dropper, transfer about 3 drops of the heated starch solution into a well plate and then add 2 drops of iodine solution.

4. If the solution gives turns blue with iodine solution, continue heating by repeating step 2-4 till the solution no longer gives a blue color with iodine solution, stop heating and record the time needed for the completion of hydrolysis in table 8.3 in the report sheet

   Note: Rinse the dropper very thoroughly before each test.

Report Sheet 1

Date

Grade

Group Names:

Table 8.2: Part I: Reducing or Non-reducing Carbohydrates

Substance	Reducing or non-reducing carbohydrates
Glucose	
Fructose	
Sucrose	
Lactose	
Starch	

Table 8.3: Hydrolysis of Carbohydrates

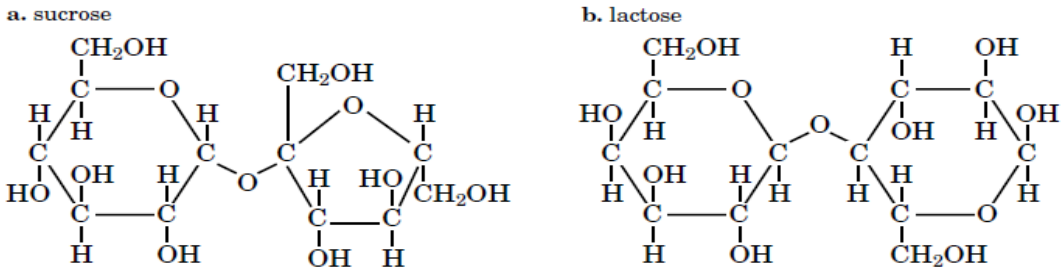
Hydrolysis of sucrose (acid versus base catalysis)		
Sample	**Condition of hydrolysis**	**Fehling's reagent (positive or negative)**
1	Acidic (H_2SO_4)	
2	Basic (NaOH)	
Hydrolysis of starch (enzyme versus acid catalysis)		
Sample	**Condition of hydrolysis**	**Iodine test (positive or negative)**
1	Enzymatic (saliva)	
2	Acidic (H_2SO_4)	
Acid catalyzed hydrolysis of starch		
Sample	**Heating time (min.)**	**Iodine test (positive or negative)**
1	5	
2	10	
3	15	
4	20	

Report Sheet 2

Answer the following questions:

1. Sucrose is a non-reducing sugar. After complete acid hydrolysis, will there be reducing groups? How many per sucrose molecule?

2. Circle and label the hemiacetal functional group and the acetal functional group in the following carbohydrates:



3. When a reducing sugar reacts with Fehling's reagent, what will be the product besides Cu_2O?

Experiment 9

Hydrolysis of Starch a Polysaccharide

Objectives

To experience the enzyme-catalyzed and acid-catalyzed hydrolysis of polysaccharides

Introduction

Starch is a complex carbohydrate of high molecular weight having the composition (C6H10O5)n. It is known to be composed of glucose units, for this is the sole monosaccharide obtained on complete hydrolysis of the material by means of hydrochloric acid. Under the influence of certain enzymes hydrolysis proceeds just as easily as with a mineral acid but not as far. The enzyme diastase, which is present in malt, brings about hydrolysis to maltose. The saliva contains an enzyme, ptyalin, which likewise converts starch largely into maltose. This disaccharide is susceptible to hydrolysis by hydrochloric acid and hence cannot be isolated in the reaction of starch with this acid although it undoubtedly is an intermediate product. It is probable that starch is made up of a series of maltose units linked together by oxygen bridges by a process akin to a polymerization.

Starch occurs in plants in the form of granules and it is not directly soluble in water. When it is boiled with water the granules swell and the material gradually passes into solution, possibly as the result of a partial depolymerization

Even soluble starch must consist of fairly large molecules, for the solution is colloidal. This can be shown clearly by a simple dialysis experiment. If a solution containing both starch and glucose is placed in a parchment bag and this is left suspended in pure water for some hours, tests will show that glucose has diffused through the parchment and that the starch has not.

Starch is easily recognized, and at the same time distinguished from the other carbohydrates, by the characteristic blue color of some product of unknown nature which is formed by reaction with iodine. The test is very useful in following the progress of a dialysis or-a hydrolysis. In the latter case an intermediate stage is reached at which a red color is produced with iodine.

At this point the solution contains a certain amount of substances known as dextrins. These are probably tetra- or pentasaccharides, but their exact nature is unknown; a mixture of such products (" dextrin ") is used as a mucilage.

Materials

Table 9.1: Chemicals and Supplies

Chemicals	Supplies
Starch	Boiling water bath and Ice-water bath
I_2/KI solution	1 small test tube
5 mL Saliva (Volunteer from each group)	100 mL Beaker (2) and 250 mL Beaker
Conc. HCl	10 mL Graduated cylinder
	Thermometer and glass rod
	Well-plate and dropper

***** ***TA must prepare*** I_2/KI solution by dissolving 1 g of iodine in a solution of 2 g KI in 8 mL of H_2O in a beaker.

Procedure

1. Measure 100 mL of distilled water and transfer it to a 250 mL beaker and heat to boiling.

2. Prepare colloidal solution of starch by mixing thoroughly 1 gram of starch with 10 mL of distilled water in a 100 mL beaker until a uniform paste is obtain

3. Transfer the paste with stirring to the beaker in step 1 to make starch solution.

4. Take 1 mL from solution in step 3 and transfer it to a small test tube, cool it using the ice water bath then add 1 drop of I_2/KI solution.

   Note: step 4 is a test to make sure that there is no reducing sugars present prior to the reaction.

5. Divide the starch solution from step 3 into two parts. Cool the first part to 40°C and add 5 mL of saliva, stir it well.

6. Record the time and test for starch at 1 minute intervals by transferring a few drops of the starch solution from the first part and add 1 drop of I_2/KI solution.

   Note: observe any change in the appearance of the solution and record the time of noting such change, this will represent the time of a complete hydrolysis

7. To the second part of the starch solution from step 5, add 1 mL of conc. HCl and heat the solution using the boiling water bath.

8. Make test for starch for every 5 minutes interval by repeating I_2/KI test in step 6

   Note: observe any change in the appearance of the solution and record the time of noting such change, this will represent the time of a complete hydrolysis.

Report Sheet 1

Grade

Group Names:

Date:

Table 9.2: Hydrolysis of Starch

enzyme catalyzed hydrolysis of starch		
Part 1	**Condition of hydrolysis**	**Iodine test (positive or negative)**
	Enzymatic (saliva)	
Sample	**Cooling time (min.)**	**Iodine test (positive or negative)**
1	1	
2	2	
3	3	
4	4	
Acid catalyzed hydrolysis of starch		
Part 2	**Condition of hydrolysis**	**Iodine test (positive or negative)**
	Acidic (HCl)	
Sample	**Heating time (min.)**	**Iodine test (positive or negative)**
1	5	
2	10	
3	15	
4	20	

Report Sheet 2

Answer the following questions:

1. The hydrolysis of starch was stopped when the iodine test no longer gave a blue color. Does this mean that the starch solution was completely hydrolyzed to glucose? Explain.

2. Which hydrolysis of the starch is faster? On the basis of this experiment estimate what will happen to the digestion of a piece of bread (containing starch) when you chew it thoroughly?

Experiment 10

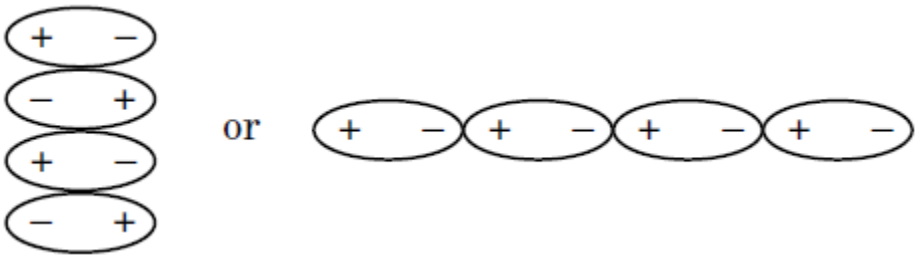
Isolation and Identification of Casein (Protein)

Objectives

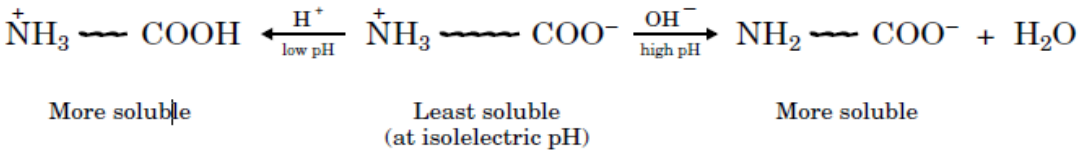
1. To isolate the casein from milk under isoelectric conditions.
2. To perform some chemical tests to identify proteins.

Introduction

Casein is the most important protein in milk. It functions as a storage protein, fulfilling nutritional requirements. Casein can be isolated from milk by acidification to bring it to its isoelectric point. At the isoelectric point, the number of positive charges on a protein equals the number of negative charges. Proteins are least soluble in water at their isoelectric points because they tend to aggregate by electrostatic interaction. The positive end of one protein molecule attracts the negative end of another protein molecule, and the aggregates precipitate out of solution.



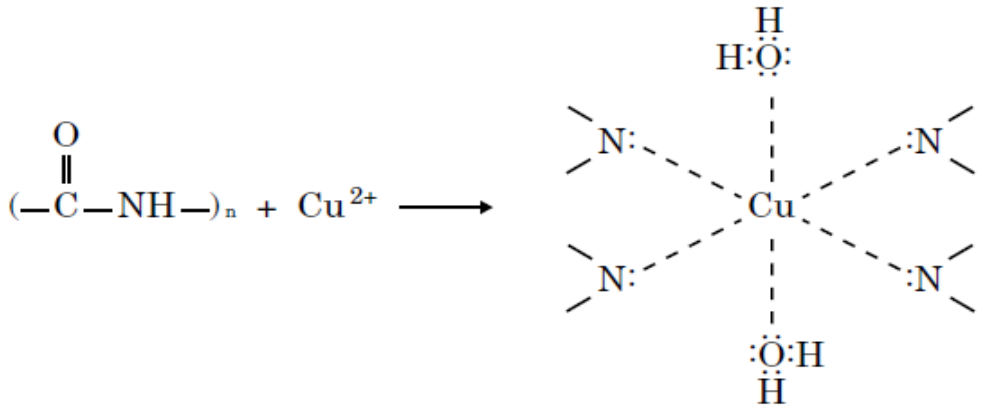
On the other hand, if a protein molecule has a net positive charge (at low pH or acidic condition) or a net negative charge (at high pH or basic condition), its solubility in water is increased.



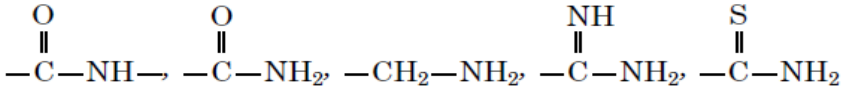
In the first part of this experiment, you are going to isolate casein from milk which has a pH of about 7. Casein will be separated as an insoluble precipitate by acidification of the milk to its isoelectric point (pH = 4.6). The fat that precipitates along with casein can be removed by dissolving it in alcohol.
In the second part of this experiment, you are going to prove that the precipitated milk product is a protein.

The identification will be achieved by performing a few important chemical tests.

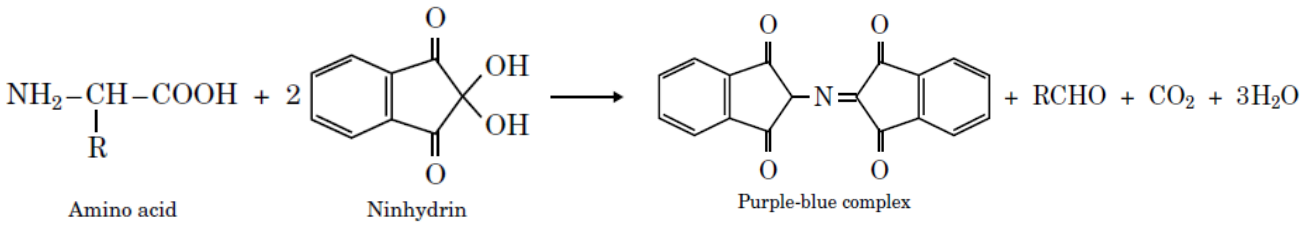
1. ***The biuret test.*** This is one of the most general tests for proteins. When a protein reacts with copper(II) sulfate, a positive test is the formation of a copper complex which has a violet color.



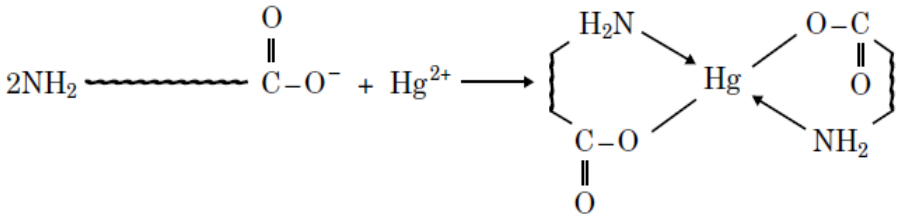
This test works for any protein or compound that contains two or more of the following groups:



2. ***The ninhydrin test.*** Amino acids with a free -NH_2 group and proteins containing free amino groups react with ninhydrin to give a purple-blue complex.



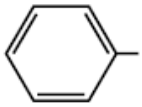
3. ***Heavy metal ions test.*** Heavy metal ions precipitate proteins from solution. The ions that are most commonly used for protein precipitation are Zn^{2+}, Fe^{3+}, Cu^{2+}, Sb^{3+}, Ag^{+}, Cd^{2+}, and Pb^{2+}. Among these metal ions, Hg^{2+}, Cd^{2+}, and Pb^{2+} are known for their notorious toxicity to humans. They can cause serious damage to proteins (especially enzymes) by denaturing them. This can result in death. The precipitation occurs because proteins become cross-linked by heavy metals as shown below:



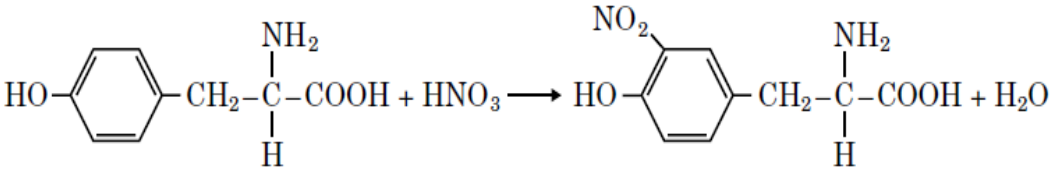
Insoluble precipitate

Victims swallowing Hg^{2+} or Cd^{2+} ions are often treated with an antidote of a food rich in proteins, which can combine with mercury or lead ions in the victim's stomach and, hopefully, prevent absorption! Milk and raw egg white are used most often. The insoluble complexes are then immediately removed from the stomach by an emetic.

4. ***The xanthoprotein test.*** This is a characteristic reaction of proteins that contain phenyl rings



Concentrated nitric acid reacts with the phenyl ring to give a yellow-colored aromatic nitro compound. Addition of alkali at this point will deepen the color to orange.



Tyrosine Colored compound

The yellow stains on the skin caused by nitric acid are the result of the xanthoprotein reaction.

Materials

Table 10.1: Chemicals and Supplies

Chemicals	Supplies
Liquid milk	250 mL Erlenmeyer flask
Glacial acetic acid	600 mL beaker serve as boiling water bath
Ethanol	Thermometer and stirring rod
1:1 mixture of diethyl ether-ethanol	Cheese cloth
2% glycine	Rubber band
2% gelatin	100 mL beaker (2)
2% albumin	5 small test tubes
1% Tyrosine	Hot plate
10% NaOH	
1 M $CuSO_4$	
Ninhydrin reagent	
$Pb(NO_3)_2$	
$Hg(NO_3)_2$	
$NaNO_3$	
Conc HNO_3	

Note: TA must prepare the necessary solutions before lab starts.

Procedure

Part I: Isolation of Casein

1. Heat in a 600-mL beaker about 200 mL of tap water till boiling.

2. Put a 250-mL Erlenmeyer flask on the scale, tare it, and weigh 50 g of milk.

3. Immerse the Erlenmeyer flask in the water bath you prepared in step 1 and Stir the solution constantly with a stirring rod. See Fig. 10.1.

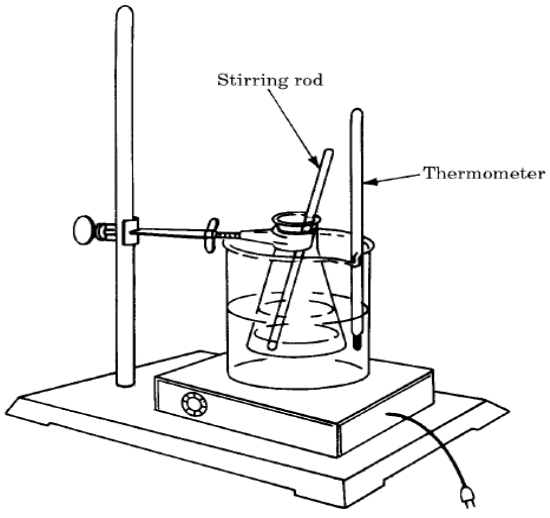


Figure 10.1: Precipitation of Casein

4. When the bath temperature has reached about 40°C, remove the flask from the water bath, and add about 10 drops of glacial acetic acid while stirring. Observe the formation of a precipitate.

5. Filter the mixture into a 100-mL beaker by pouring it through a cheese cloth which is fastened with a rubber band over the mouth of the beaker (Fig. 10.2).

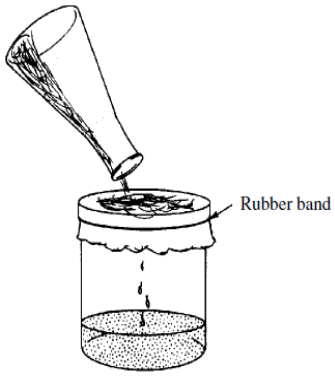


Figure 10.2: Filtration of Casein

6. Remove most of the water from the precipitate by squeezing the cloth gently.

7. Discard the filtrate in the beaker. Using a spatula, scrape the precipitate from the cheese cloth into the empty clean beaker.

8. Add 25 mL of ethanol to the beaker in step 7. After stirring the mixture for 5 min., allow the solid to settle.

9. Carefully decant the liquid that contains fats into another beaker to discard this liquid.

10. To the residue (solid), add 25 mL of a 1:1 mixture of diethyl ether-ethanol. After stirring the resulting mixture for 5 min., collect the solid by vacuum filtration, refer to figure 5.1.

11. Spread the casein on a paper towel and let it dry.

12. Weigh the dried casein and calculate the percentage of casein in the milk. Record it in the report sheet.

Part II: Chemical Analysis of Proteins

A. The biuret test

1. Label 4 clean test tubes as glycine, gelatin, albumin, and Tyrosine respectively then add 15 drops of each sample to be tested.

2. In test tube no. 5 put a small amount of Casein prepared in PartI and add 15 drops of distilled water.

   To each of the test tubes, add 5 drops of 10% NaOH solution and 2 drops of 1M CuSO4 solution with stirring. Record your observation in table 10.2 in the report sheet.

   Note: Observing a purplish-violet color is an evidence of the presence of proteins.

B. The ninhydrin test

1. Repeat steps 1 and 2 from part II (A).

2. To each of the test tubes, add 5 drops of ninhydrin reagent

3. Heat the test tubes in a boiling water bath you prepared in part I (step 1) for about 5 min and record your observation in table 10.2 in the report sheet.

C. Heavy metal ions test

1. Label 3 clean test tubes as no.1, no. 2, and no. 3.

2. Add 2 mL of liquid milk in each test tube.

3. Add a few drops of each of the following metal ions to the corresponding test tubes as indicated below and record your observation in table 10.2 in the report sheet
   a. Pb^{2+} as $Pb(NO_3)_2$ to test tube no.1
   b. Hg^{2+} as $Hg(NO_3)_2$ to test tube no.2
   c. Na^{+} as $NaNO_3$ to test tube no.3

D. The xanthoprotein test.

1. Repeat steps 1 and 2 from part II (A).

2. To each of the test tubes, add 10 drops of concentrated HNO_3 while swirling.

3. Heat the test tubes carefully in a warm water bath. Observe any change in color and record your observation in table 10.2 in the report sheet

Report Sheet 1

Grade

Group Names:

Date

Part I: Isolation of Casein

1. Weight of milk _____ g

2. Weight of dried casein _____ g

3. $$\% \text{ casein} = \frac{\text{weight of solid (casein)}}{50.00 \text{ g of milk}} \times 100$$

Percent of casein = _____ %

Table 10.2 ***Part II: Chemical analysis of proteins***

Samples	**A. Biuret test**	**B. Ninhydrin test**	**C. Heavy metal ion test**	**D. Xanthoprotein test**
	Color formed	**Color formed after heating**	**Precipitates formed**	**Color formed before or after heating**
2% glycine				
2% gelatin				
2% albumin				
casein + H_2O				
1% tyrosine				

A. Which of these chemicals gives a positive test with **Biuret** reagents? ----------

B. Which of these chemicals gives a positive test with **Ninhydrin** reagents? ----------

C. Which of these metal ions gives a positive test with casein in milk? ----------

D. Which of these chemicals gives a positive test with **Xanthoprotein** reagent? ----------

Report Sheet 2

Answer the following questions:

1. Casein has an isoelectric point at pH 4.6. What kind of charges will be on the casein in its native environment, that is, in milk?

2. Would the amino acid, glycine, give a positive biuret test? Explain

3. What are the three most toxic heavy metal ions?

Experiment 11

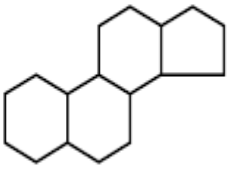
Analysis of Lipids

Objectives

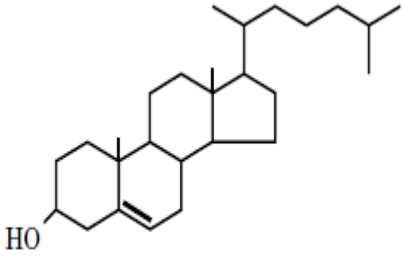
To investigate the lipid composition of common foods such as corn oil, butter, and egg yolk.

Introduction

Lipids are chemically heterogeneous mixtures. The only common property they have is their insolubility in water. We can test for the presence of various lipids by analyzing their chemical constituents. Foods contain a variety of lipids, most important among them are fats, complex lipids, and steroids. Fats are triglycerides, esters of fatty acids and glycerol.
Complex lipids also contain fatty acids, but their alcohol may be either glycerol or sphingosine. They also contain other constituents such as phosphate, choline, or ethanolamine or mono- to oligo-saccharides. An important representative of this group is lecithin, a glycerophospholipid, containing fatty acids, glycerol, phosphate, and choline.
The most important steroid in foods is cholesterol. Different foods contain different proportions of these three groups of lipids.
Structurally, cholesterol contains the steroid nucleus that is the common core of all steroids.



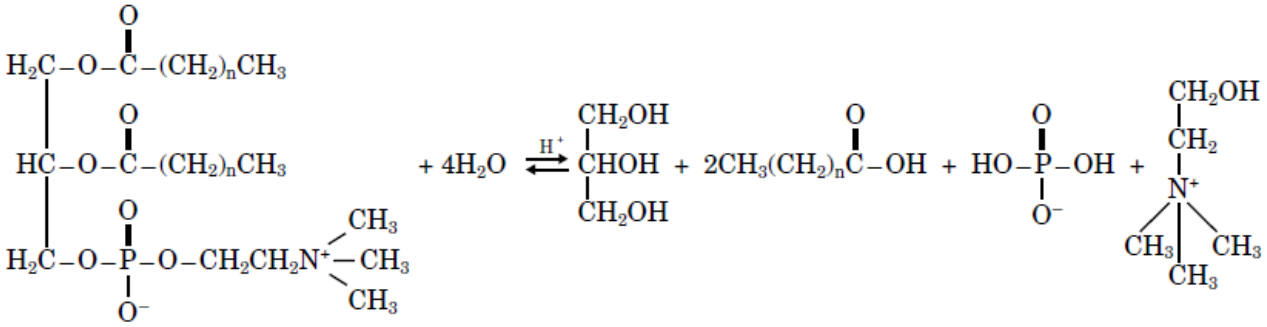
Steroid nucleus



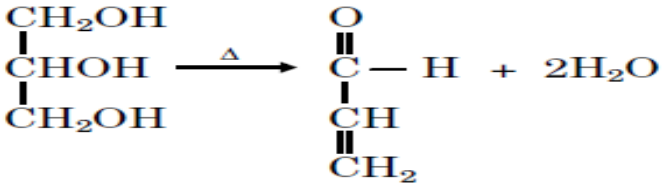
Cholesterol

There is a special colorimetric test, the Lieberman-Burchard reaction, which uses acetic anhydride and sulfuric acid as reagents, that gives a characteristic green color in the presence of cholesterol. This color is due to the -OH group of cholesterol and the unsaturation found in the adjacent fused ring. The color change is gradual: first it appears as a pink coloration, changing later to lilac, and finally to deep green.

When lecithin is hydrolyzed in acidic medium, both the fatty acid ester bonds and the phosphate ester bonds are broken and free fatty acids and inorganic phosphate are released. Using a molybdate test, we can detect the presence of phosphate in the hydrolysate by the appearance of a purple color. Although this test is not specific for lecithin (other phosphate containing lipids will give a positive molybdate test), it differentiates clearly between fat and cholesterol on the one hand (negative test), and phospholipid on the other (positive test).



A second test that differentiates between cholesterol and lecithin is the acrolein reaction. When lipids containing glycerol are heated in the presence of potassium hydrogen sulfate, the glycerol is dehydrated, forming acrolein, which has an unpleasant odor. Further heating results in polymerization of acrolein, which is indicated by the slight blackening of the reaction mixture. Both the pungent smell and the black color indicate the presence of glycerol, and thereby fat and/or lecithin. Cholesterol gives a negative acrolein test.



Materials

Table 11.1: Chemicals and Supplies

Chemicals	Supplies
0.2 g pure cholesterol	6 large test tubes
0.2 g pure glycerol	6 small test tubes
0.2 g lecithin	250 mL beaker serve as boiling water bath
Corn oil	100 mL beaker (2)
Butter	Cheese cloth
Egg yolk	250 mL Erlenmeyer flask
6 M HNO_3	Hot plate
6 M NaOH	
Molybdate solution	
Ascorbic acid	
$KHSO_4$	
Sucrose	
Chloroform	
Acetic anhydride	
Conc. H_2SO_4	

Procedure

Part I: Phosphate Test

1. Prepare a water bath by boiling about 100 mL of tap water in a 250-mL beaker on a hot plate.

2. Label 6 clean test tubes as cholesterol, glycerol, lecithin, Corn oil, Butter and Egg yolk respectively then add 0.2 g of the sample to each test tube to be tested.

3. Hydrolyze the compounds by adding 3 mL of 6 M nitric acid to each test tube

4. Place the test tubes in the boiling water bath for 5 min. after that leave them to cool down.

   Note: Do not inhale the vapors.

5. Neutralize the acid by adding 3 mL of 6 M NaOH. Mix. During the hydrolysis, a precipitate may form, especially in the egg yolk sample.

6. The samples in which a precipitate appeared must be filtered. Place a piece of cheese cloth on top of a 100-mL beaker. Pour the turbid hydrolysate in the test tube on the cheese cloth and filter it. Figure 10.2

7. Transfer 2 mL of each neutralized (and filtered) sample into small clean and labeled test tubes. Add 3 mL of a molybdate solution to each test tube and mix the contents.

8. Heat the test tubes in a boiling water bath for 5 min. cool them to room temperature.

9. Add 0.5 mL of an ascorbic acid solution and mix the contents thoroughly.

10. Wait 20 min. for the development of the purple color. Record your observations in table 11.2 in the report sheet.

Part II: The Acrolein Test for Glycerol

1. Label 7 clean test tubes as cholesterol, glycerol, lecithin, Corn oil, Butter, Egg yolk and sucrose respectively then add 0.1 g of the sample to each test tube to be tested.

2. Add 1 g of $KHSO_4$, in each of seven test tubes

Note: It is important that step 3 test be performed under the hood because of the pungent odor of the acrolein.

3. Set up your Bunsen burner in the hood and gently heat each test tube, one at a time, over the Bunsen burner flame, shaking it continuously from side to side. When the mixture melts it slightly blackens, and you will notice the evolution of fumes. Stop the heating. A pungent odor, resembling burnt hamburgers, is the positive test for Glycerol. Sucrose also will be dehydrated and will give a black color. However, its smell is different, and thus is not a positive test for acrolein.

Note1: Smell the test tubes by moving them sideways under your nose or waft the vapors. Do not inhale the fumes directly.

Note2: Do not overheat the test tubes, for the residue will become hard, making it difficult to clean the test tubes. Record your observations on the Report Sheet.

Part III: Lieberman-Burchard Test for Cholesterol

1. Repeat step 1 in part II

Note: The next step should be done in

2. Add 3 mL of chloroform and 1 mL of acetic anhydride to each test tube.

3. Add 1 drop of conc. H_2SO_4 to each mixture. Mix the contents and record the color changes, if any. Wait 5 min. Record again the color of your solutions in table 11.2 in the report sheet.

Report Sheet 1

Date

Grade

Group Names:

Table 11.2: Record observation

Tests	Cholesterol	Lecithin	Glycerol	Corn oil	Butter	Egg yol	Sucrose
1. Phosphate a. color							
b. Conclusions							
2. Acrolein a. Odor							
b. Color							
c. Conclusions							
3. Lieberman-Burchard **a.** Initial color							
b. Color after 5 min.							
c. Conclusion							

Report Sheet 2

Answer the following questions:

1. Based on the intensity of color developed in your test for cholesterol, which food contained the most and which contained the least cholesterol?

2. Cholesterol in tissues is sometimes esterified by fatty acids. (a) Draw the structure of cholesterol oleate. (b) Would this ester give a positive Lieberman-Burchard test?

3. Why was it necessary to hydrolyze the samples with nitric acid before performing the molybdate test?

4. Why must you wear gloves in performing the phosphate test?

6. References

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PERIODIC TABLE OF THE ELEMENTS

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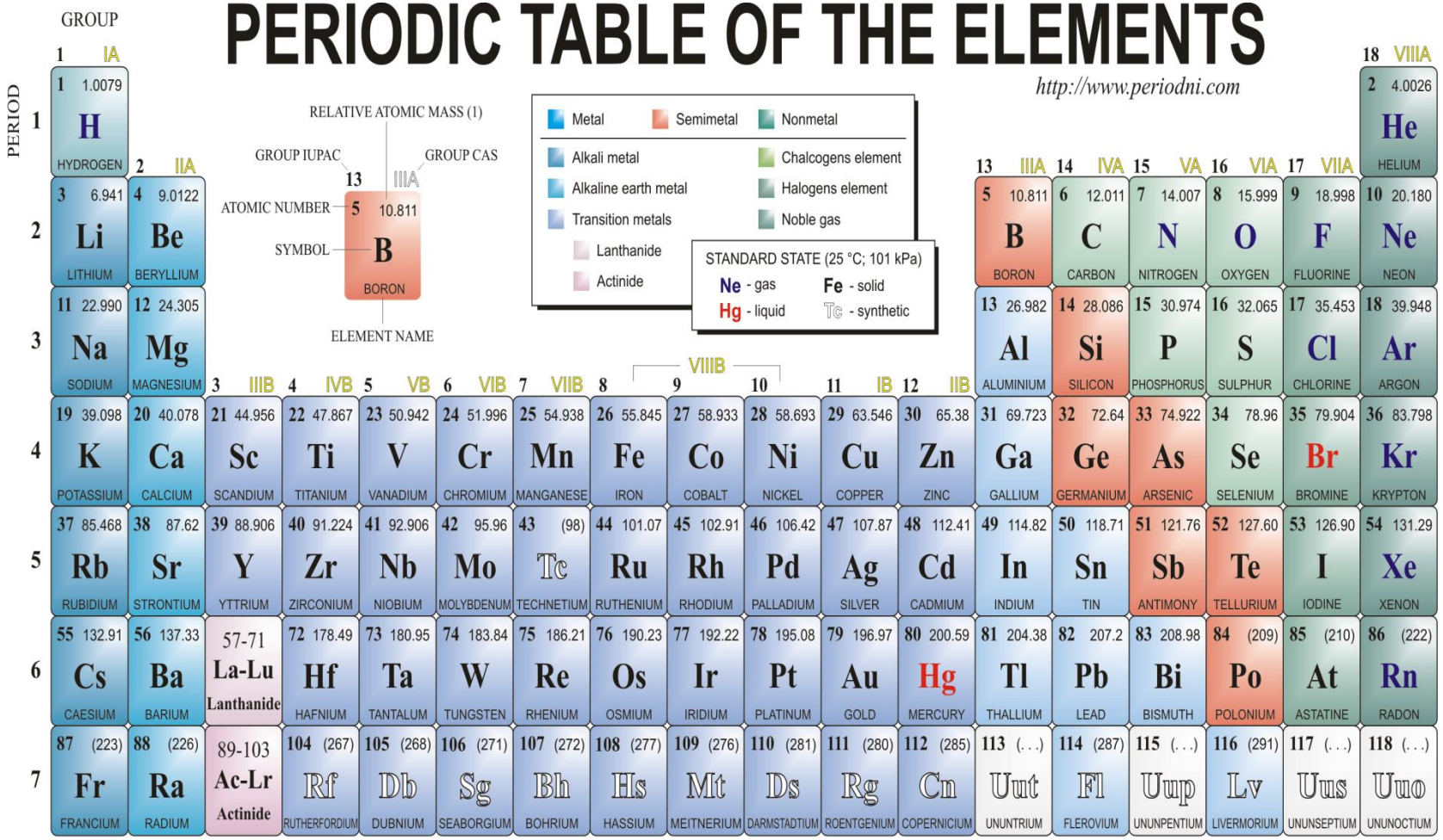

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(1) Pure Appl. Chem., **81**, No. 11, 2131-2156 (2009)
Relative atomic masses are expressed with five significant figures. For elements that have no stable nuclides, the value enclosed in brackets indicates the mass number of the longest-lived isotope of the element. However three such elements (Th, Pa and U) do have a characteristic terrestrial isotopic composition, and for these an atomic weight is tabulated.