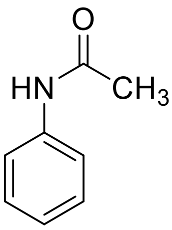
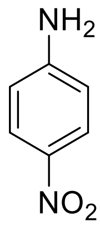
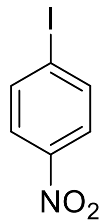
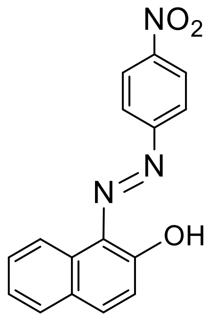
Organic Laboratory 2

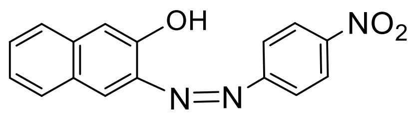
คม254: ปฏิบัติการเคมีอินทรีย์ 2

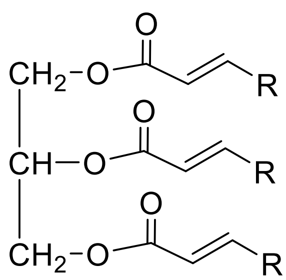


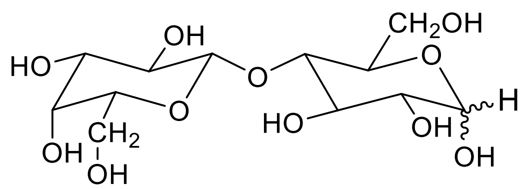












สาขาวิชาเคมี คณะวิทยาศาสตร์

มหาวิทยาลัยแม่โจ้

ประจำปีการศึกษา 2/2564

Contents

หลักสูตรวิทยาศาสตรบัณฑิต สาขาวิชาเคมี คณะวิทยาศาสตร์ มหาวิทยาลัยแม่โจ้
ประมวลการสอนรายวิชา (Course Syllabus)
ประจำปีการศึกษาที่ 2564 ภาคเรียนที่ 2

รหัสวิชา คม254 **ชื่อรายวิชา** ปฏิบัติการเคมีอินทรีย์ 2
จำนวนหน่วยกิตรวม 3 หน่วยกิต [ปฏิบัติการ 3 ชั่วโมง/สัปดาห์]

คำอธิบายรายวิชา

การศึกษาเทคนิคปฏิบัติการ ปฏิกิริยาเคมีและการสังเคราะห์สมัยใหม่ กลไก การแยก และการระบุเอกลักษณ์ของสารอินทรีย์ ของสารประกอบคาร์บอนิล อัลดีไฮด์ คีโตน เอมีน แอโรมาติก กรดคาร์บอกซิลิก อนุพันธ์ ฟีนอล คาร์โบไฮเดรต กรดอะมิโน และไขมัน

วัตถุประสงค์รายวิชา

CLO1 นักศึกษาสามารถเข้าใจการสังเคราะห์และกลไกการเกิดปฏิกิริยาของ Electrophilic Aromatic Substitution Nitration, Nucleophilic Aromatic Substitution with KI Diazonium salt Coupling reaction with β-naphthol ของสารอะโรมาติกได้

CLO2 นักศึกษาสามารถสังเคราะห์สารและเข้าใจกลไกการเกิดปฏิกิริยาของ The Aldol condensation และ The Claisen condensation ได้

CLO3 นักศึกษาสามารถสังเคราะห์สารและเข้าใจกลไกการเกิดปฏิกิริยาของ Synthesis of carboxylic derivative; Aspirin Cannizzaro Reaction of benzaldehyde

CLO4 นักศึกษาสามารถยืนยันโครงสร้างสารด้วยเทคนิค FT-IR ของผลิตภัณฑ์จากปฏิกิริยา Cannizzaro ได้

CLO5 นักศึกษามีคุณธรรม จริยธรรม และจรรยาบรรณในการวิจารณ์ผล สรุปผลการทดลอง และการเขียนรายงานปฏิบัติการ

อาจารย์ผู้สอน

อ.ดร. ปิยธิดา กล่ำภู่ section 1 วันจันทร์ 15.00-18.00 น.

แผนที่แสดงการกระจายความรับผิดชอบมาตรฐานผลการเรียนรู้สู่รายวิชา (Curriculum Mapping)

วิทยาศาสตร์และคณิตศาสตร์

รายวิชา	คุณธรรมและจริยธรรม					ความรู้				ทักษะทางปัญญา			ทักษะระหว่างบุคคลและความรับผิดชอบ			ทักษะวิเคราะห์เชิงตัวเลข การสื่อการ และการใช้เทคโนโลยีสารสนเทศ			
	1	2	3	4	5	1	2	3	4	1	2	3	1	2	3	1	2	3	4
คม254 ปฏิบัติการเคมีอินทรีย์ 2	●	●	○			●	●			●	○		●	●		●	○		○

รายละเอียดการสอนของเนื้อหาวิชา

สัปดาห์ #	บท #	บท/หัวข้อ/เรื่อง	จำนวนชั่วโมง	ผู้สอน
1-3		แนะนำห้องปฏิบัติการ ข้อควรปฏิบัติและความปลอดภัยในห้องปฏิบัติการ แนะนำและอธิบายวิธีการเขียนรายงานการทดลอง การเก็บข้อมูล การคำนวณ การสรุปผล และการอภิปรายผลการทดลองของทุกปฏิบัติการ **(Online)**	15	อ.ดร.ปิยธิดา
4	**1**	ปฏิบัติการที่ 1 Electrophilic Aromatic Substitution (Nitration reaction)	3	อ.ดร.ปิยธิดา
5	**2**	ปฏิบัติการที่ 2 Nucleophilic Aromatic Substitution with KI	3	อ.ดร.ปิยธิดา
6	**3**	ปฏิบัติการที่ 3 Diazonium salt Coupling reaction with β-naphthol	3	อ.ดร.ปิยธิดา
7	4	ปฏิบัติการที่ 4 Synthesis of carboxylic derivative; Aspirin	3	อ.ดร.ปิยธิดา
8	**5**	ปฏิบัติการที่ 5 The Aldol condensation	3	อ.ดร.ปิยธิดา
สอบกลางภาค 2/2564 (17-23 มกราคม 2565) **งดปฏิบัติการ**				
9	**6**	ปฏิบัติการที่ 6 The Claisen condensation	3	อ.ดร.ปิยธิดา
10	**7**	ปฏิบัติการที่ 7 Cannizzaro Reaction of benzaldehyde	3	อ.ดร.ปิยธิดา
11	**8**	ปฏิบัติการที่ 8 Identification of Cannizzaro Reaction Product by FT-IR technique	3	อ.ดร.ปิยธิดา
12-14	**9**	ทบทวนบทเรียน ส่งรายงาน สอบเทคนิคปฏิบัติการ	3	อ.ดร.ปิยธิดา
15	**10**	เช็คและคืนอุปกรณ์ ตรวจเช็คคะแนนรายงาน	3	อ.ดร.ปิยธิดา
สอบปลายภาค 2/2564 (14-27 มีนาคม 2565)				

เกณฑ์การให้คะแนน

การประเมินผล	สัดส่วน
แบบทดสอบก่อนเรียน	5%
แผนการทดลองและบันทึกผลการทดลอง	5%
สังเกตพฤติกรรมในห้องปฏิบัติการ	5%
รายงานผลการทดลอง	50%
สอบเทคนิคปฏิบัติการ	5%
สอบข้อเขียนปลายภาค	30%
รวมทั้งสิ้น	**100 %**

เกณฑ์การตัดเกรด

45 + 3.5SD % ขึ้นไป	ระดับคะแนน A	45 + 1.5SD %	ระดับคะแนน C
45 + 3.0SD %	ระดับคะแนน B+	45 + 1.0SD %	ระดับคะแนน D+
45 + 2.5SD %	ระดับคะแนน B	45+ 0.5SD %	ระดับคะแนน D
45 + 2.0SD %	ระดับคะแนน C+	ต่ำกว่า 45 %	ระดับคะแนน F

ตำราและเอกสารประกอบการสอน

1. *คู่มือปฏิบัติรายวิชา คม 252 ปฏิบัติการเคมีอินทรีย์ 2*, คณะวิทยาศาสตร์, มหาวิทยาลัยแม่โจ้, 2564.

2. *VOGEL's Textbook of practical organic chemistry* 5th ed. Brian S. Furniss; Antony J. Hannaford; Peter W. G. Smith; Austin R. Tatchell. **1989**

3. *Microscale and Macroscale Techniques in the Organic Laboratory*. Domald L. Pavia; Gary M. Lampman; George S. Kriz; Randall G. Engel. **2001**

4. *Modern Organic Synthesis in the Laboratory: A Collection of Standard Experimental Procedures*. Jie Jack Li; Chris Limberakis; Derek A. Pflum. **2007**

การเขียนรายงาน

เรื่อง..

วันที่ทดลอง............................กลุ่มที่...................

ผู้เขียนรายงาน ชื่อ) .... รหัส...........................สาขา................................

ผู้ร่วมงาน ชื่อ).......................................รหัส............................สาขา................................

ชื่อ).......................................รหัส..........................สาขา...............................

<u>วัตถุประสงค์</u>

ให้บอกวัตถุประสงค์ของการทดลองให้ชัดเจนว่า เพื่อต้องการหาค่าอะไร ของสารอะไร โดยวิธีไหน (ตามวิธีทำการทดลองจริง)

<u>ทฤษฎี (หลักการ)</u> ไม่เกิน 1 หน้ากระดาษ

ให้อธิบายทฤษฎีหรือหลักการโดยย่อ พร้อมทั้งสูตรและสัญลักษณ์ ในการทดลองหรือการคำนวณ เพื่อให้ได้ผลการทดลองตรงตามวัตถุประสงค์ที่ต้องการ

<u>วิธีการทดลอง</u>

- สารเคมี (ถ้ามี) จัดทำในรูปแบบตารางข้อมูล ดังตัวอย่าง

สารเคมี	Ethanol				
สูตร/โครงสร้าง	C_2H_5OH				
MW (g/mol)	46.07				
ปริมาณที่ใช้ (g/mL)	10 mL				
Mmol					
Equivalent					
BP (^{o}C)	78.24				
MP (^{o}C)	-114.14				
ข้อควรระวัง	Flammable				

- สมการแสดงปฏิกิริยาเคมี (ถ้ามี)
- วิธีการทดลอง อธิบายวิธีทำการทดลองโดยย่อ ตามลำดับขั้นที่ทำการทดลองในห้องปฏิบัติการ ตั้งแต่เริ่มต้นจนกระทั่งถึงขั้นสุดท้ายของการทดลอง แสดงรูปประกอบ Flow Chart

ส่วนที่ 2 ผลการทดลอง สรุปและวิจารณ์ผลการทดลอง (เขียนจากผลการทดลองจากการเรียนปฏิบัติการ)

ผลการทดลอง

จากการทดลองได้สารอะไร ลักษณะทางกายภาย เช่น สี รูปร่างผลึก ปริมาณสารที่ได้ (กรัม/มิลลิลิตร) สมบัติทางกายภาพอื่น ๆ ที่ทำการวิเคราะห์ เช่น จุดเดือด จุดหลอมเหลว การละลาย เป็นต้น

การคำนวณ

ให้แสดงวิธีการคำนวณที่สำคัญ ให้แสดงผลลัพธ์หรือค่าเฉลี่ยของผลลัพธ์ที่ได้จากการคำนวณสารกำหนดปริมาณ, %yield, %conversion และ %recovery

สรุปและวิจารณ์ผลการทดลอง

สรุปตามจุดประสงค์ ว่าได้สาร(ชื่อ) ได้สารกี่กรัม คิดเป็น ร้อยละ (%yield)

การวิจารณ์ผลการทดลอง วิจารณ์ผลว่าทำไมถึงเป็นเช่นนั้น และ ทำไมถึงไม่เป็นเช่นนั้น

อาจอิงตามจุดประสงค์การทดลอง

เอกสารอ้างอิง

เอกสารอ้างอิง อย่างน้อย 3 เอกสาร (หนังสือหรือเว็บไซต์)

Laboratory Safety and MSDS

Safety!

Organic chemistry laboratory can provide social benefit, basic scientific discoveries, and intellectual satisfaction. Chemical experiments are not just fun, But can also be very hazardous, some experiments inherently so. Complacency is often observed by experience. One often forgets that chemistry is a potentially dangerous enterprise; a cavalier attitude often results in disastrous consequences. Therefore, extreme caution should be exercised at all time, especially when one handles large-scale reactions that are exothermic or when dealing with toxic chemicals.

Personal Protection Equipment

1. Safety Glasses

If a chemical splash into your eyes, it could do serious and sometimes permanent damage to your vision. The most common forms of eye protection include safety glasses (with side shields), goggles, and face shields. Prescription eye glasses are acceptable provided that the lenses are impact resistant and they are equipped with side shields.

2. Gloves

Laboratory gloves are an essential part of safe laboratory practice and must be worn while handling chemicals. Despite practicing good safety techniques, tragedy may still strike. The types of gloves available and their recommended use. The manufacturer's description of the gloves should be consulted.

3. Laboratory Coats

Laboratory coats provide an important barrier for your clothes and, more important, your skin from chemicals. The laboratory coat should fit comfortably, have long sleeves and should be clean.

4. Material Safety Data Sheets (MSDS)

When dealing with chemicals, caution is warranted, especially with reactive chemicals, carcinogens, and toxic reagents. A useful resource is the Material Safety Data Sheets (MSDS). There are a variety of resources that can be accessed online, including the following:

MSDS Solution [www. msds.com (accessed June. 10, 2013), MSDS online [www.msdsonline.com (accessed June. 10, 2013),

Cornell University [www.msds.ehs.cornell.edu (accessed June. 10, 2013),

Chemical suppliers/manufactures such as Sigma-Aldrich (www.sigmaaldrich.com),

(accessed June. 10, 2013),

www.msds.ped.go.th (accessed June. 10, 2013)

5. Useful Preparations

Setting up the reaction is probably the most import job for an organic chemist. Once a reaction is initiated, there is little left that needs to be done to change the outcome.

Experiment 1:

Electrophilic Aromatic Substitution (Nitration reaction):

Aromatic substitution reactions are generally electrophilic, due to the high electron density of the benzene ring. The species reacting with the aromatic ring is usually a positive ion or the positive end of a dipole. This electron deficient species, or electrophile, may be produced in various ways, but the reaction between the electrophile and the aromatic ring is essentially the same in all cases.

The overall directing and rate effects of a substituent can be classified into three groups:

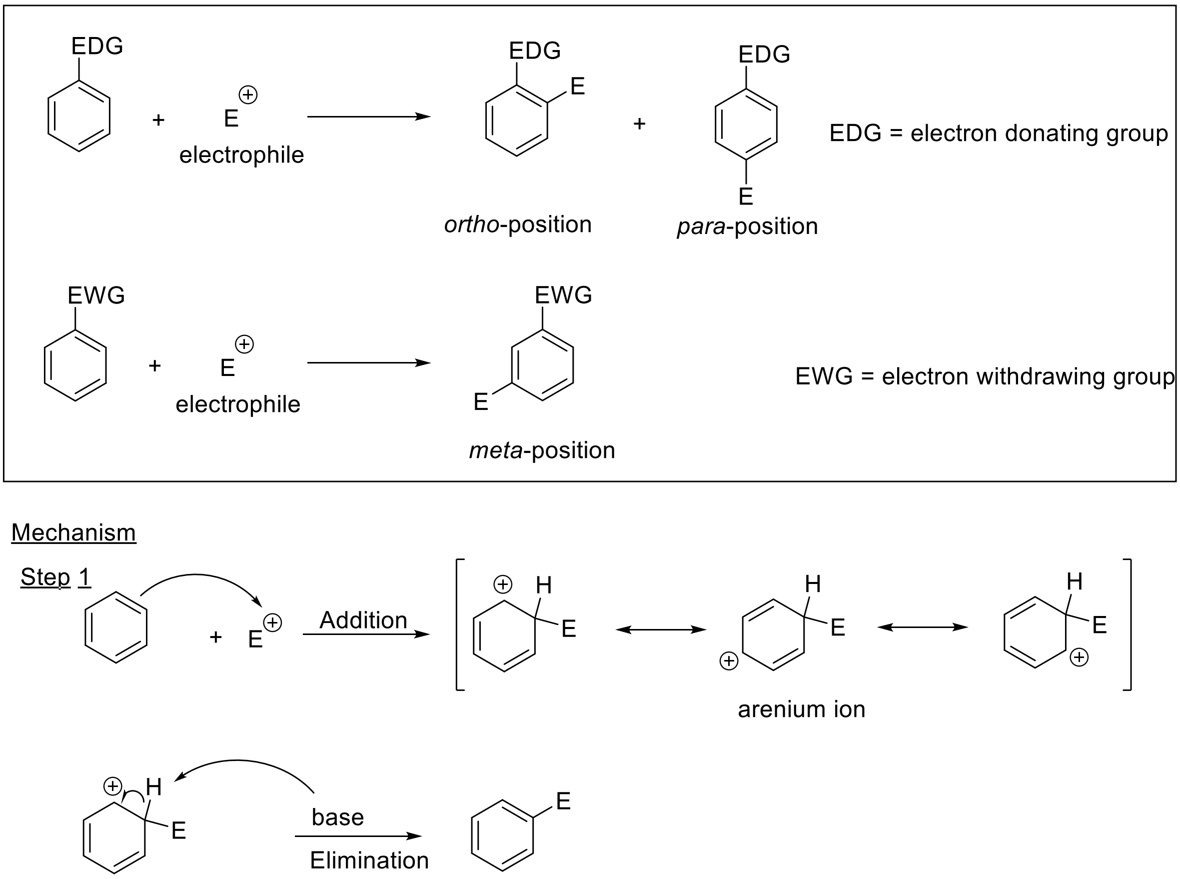
1. ortho-para-directing activators
2. ortho-para-directing deactivators
3. meta-directing deactivators.

Any substituent that activates the aromatic ring is an ortho-para director. Figure 1.1 shows the resonance forms of the arenium ion associated with a monosubstituted aromatic system. Electrophilic attack at either the ortho- or para-position placed a positive charge on the carbon. This stabilization is not possible when electrophile attacks at the meta-position

$-NH_2$, $-OCH_3$, -OH	X; halogens	$-\overset{O}{\overset{\|}{C}}-$H(R, OH, OR)
-NHCOR, $-CH_3$ (alkyl)	-F, -Br, -Cl, -I	$-NO_2$, -CN, $-SO_3H$
ortho-para activators	*ortho-para* deactivators	*meta* activators

Figure 1.1 The classification of substituent aromatic

Normally, electron donating group (EDG) substituent of the aromatic ring was substituted electrophile at *ortho*- and *para*- positions. The stabilization of the positive charge can occur. In another hand, electron withdrawing group substituent of the aromatic ring was substituted electrophile at *meta*- positions.



Scheme 1.1: Electrophilic aromatic substitution and their mechanism

The most common electrophilic aromatic substitution mechanism is the arenium ion mechanism, shown in above.

<u>In the first step</u> of the reaction, benzene donates an electron pair to the electrophilic species, designated E^+. A carbocation intermediate is formed, called an arenium ion or a sigma complex. This arenium ion can be written in three resonance forms. Although the arenium ion is stabilized by these resonance forms, it is destabilized by the loss of aromatic stability (~36 - 62 kcal/mole). This aromatic stability is regained in

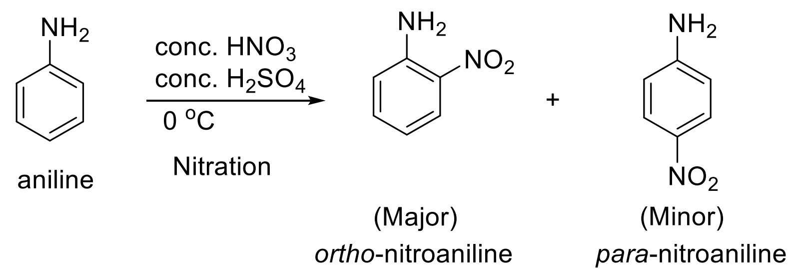
<u>the second step</u> of the reaction, consisting of elimination of a proton from the arenium ion, forming a substituted benzene. This process is called "re-aromatization".

Nitration is one of the most important examples of electrophilic aromatic substitution. Aromatic nitro compounds are used in products ranging from explosives to pharmaceutical synthetic intermediates. The electrophile in nitration is the nitronium ion (NO_2^+). The nitronium ion is generated from nitric acid by protonation and loss of water, using sulfuric acid as the dehydrating agent as shown in Scheme 1.2.

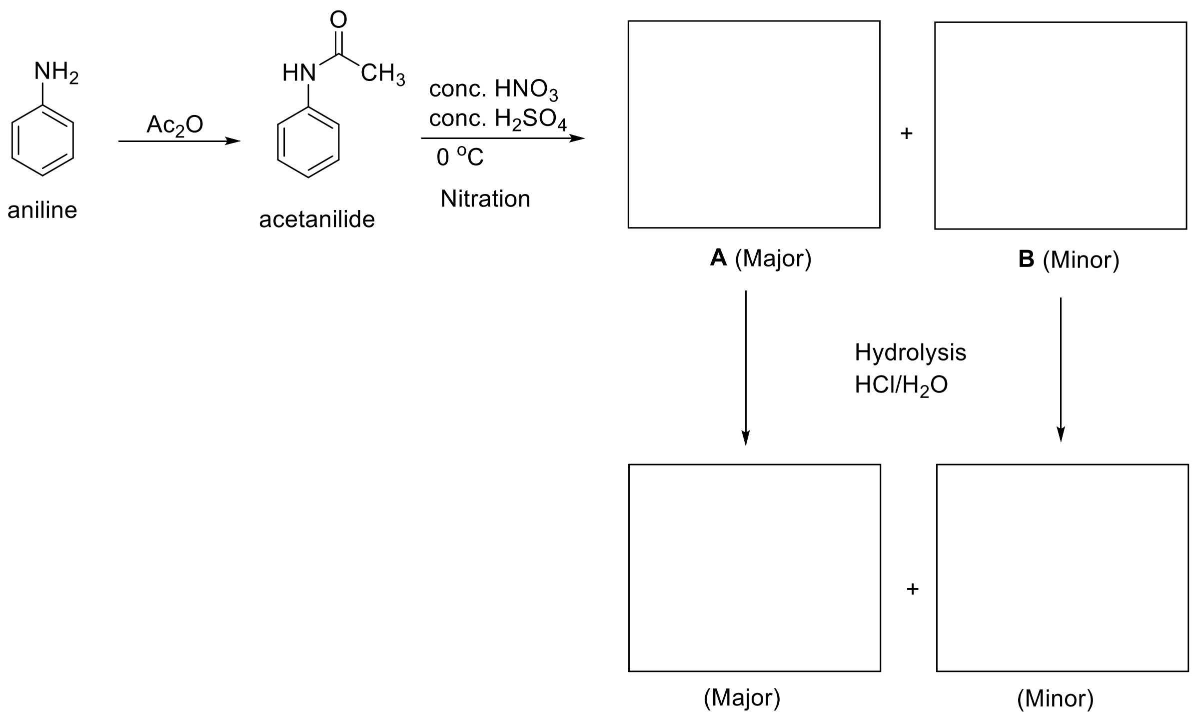
$$HNO_3 + 2\,H_2SO_4 \longrightarrow \underset{\text{nitronium ion}}{O{=}\overset{\oplus}{N}{=}O} + H_3O^{\oplus} + 2\,HSO_4^{\ominus}$$

Scheme 1.2: Preparation of nitronium ion

Nitration of aniline was give *ortho*-nitroaniline as major product and *para*-nitroaniline as minor product. In this experiment, you will perform a nitration of acetanilide to *ortho*-nitroacetanilide and *para*-nitroacetanilide products. Then, hydrolysis with acid to give *ortho*-nitroaniline and *para*-nitroaniline product.



Scheme 1.3: Nitration reaction of aniline



Scheme 1.4: Nitration reaction of acetanilide and hydrolysis reaction

Procedures

Part 1: Syntheses of Nitroacetanilide

1. Weigh 1.50 g of acetanilide and 3.0 ml of conc. H_2SO_4 in 250 ml beaker, stir on an ice bath (< 5 ^oC)
2. Slowly drop nitrating solution (1.5 ml of H_2SO_4 and 1.5 ml of HNO_3) "carefully the solution temperature must below < 5 ^oC" and stir for 10 minutes.
3. Add 100 ml of cool water into the solution. The particle of Nitroacetanilide will appear.
4. Collect the Nitroacetanilide with vacuum filtration

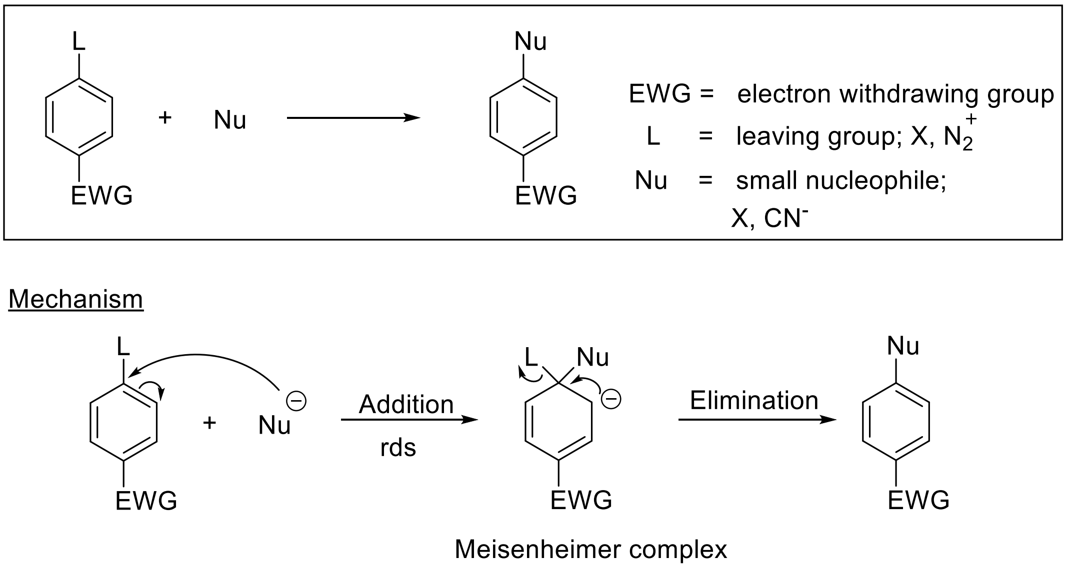
Part 2: Hydrolysis of Nitroacetanilide

1. Obtain all of Nitroacetanilide in 100 ml of round bottom flask.
2. Add 10 ml of HCl and 20 ml of water.
3. Reflux the solution for 15 minutes.
4. Decant the solution into 50 ml of cool water (beaker)
5. Basicity with 50% NaOH (Slowly drop). The particle of Nitroaniline will appear.
6. Collect the Nitroaniline with vacuum filtration and dried with oven. Record actual weight. What was your percentage yield?
7. Explain and complete Scheme 3.4, what the major and minor of your products. Why?

Experiment 2:

Nucleophilic Aromatic Substitution with KI; Diazotization reaction

Nucleophilic aromatic substitution (S_NAr) reactions offer a useful way to functionalize an aromatic ring. The high π–electron density of an aromatic ring results in predominant reactivity towards electrophiles; however, if the aromatic ring is activated with electron withdrawing groups (EWG) ortho and/or para to a good leaving group, a nucleophilic substitution reaction is possible. Halogens are the most common leaving groups for S_NAr reactions and functional groups such as $-NO_2$, $-SO_2R$, $-NR_2$, $-CF_3$ and $-CN$ are electron withdrawing enough to render the aromatic ring susceptible to reaction with an electron-rich nucleophile, such as an amine.

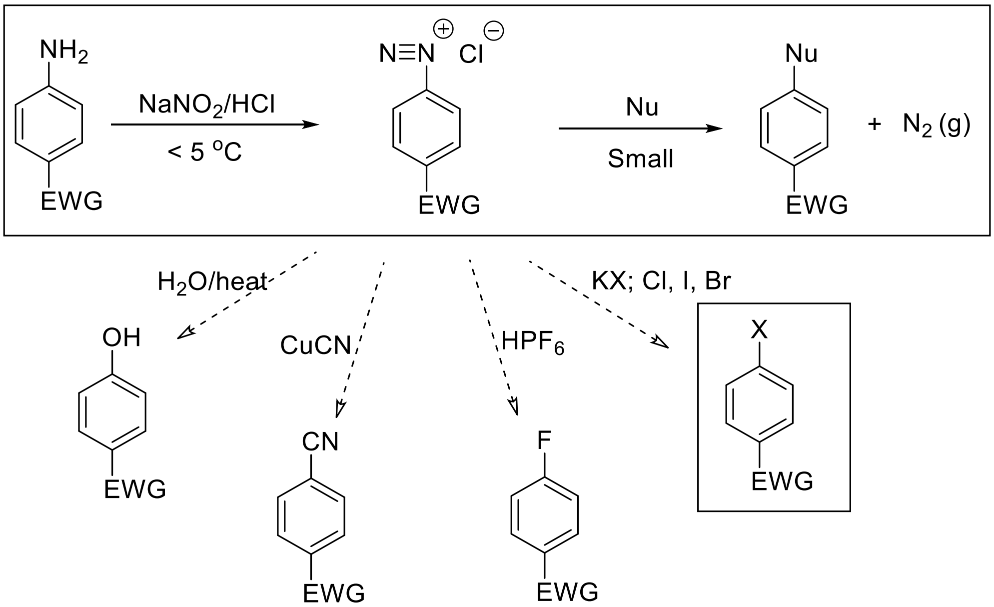


Scheme 2.1: Nucleophilic aromatic substitution and their mechanism

The reaction follows an addition-elimination two-step reaction sequence. It is generally accepted that the first step, in which a tetrahedral cyclohexadienyl anion called a Meisenheimer complex is formed, is the rate-determining step (rds). This is generated by the addition of the nucleophile to the carbon bearing the leaving group. Subsequent elimination of the halogen substituent (leaving group) leads to regeneration of the aromaticity in the ring.

Diazonium compounds or diazonium salts are a group of organic compounds sharing a common functional group $R\text{-}N^{2+}\ X^{-}$. Diazonium salts, especially those where R is an aryl group, are important intermediates in the organic synthesis.

The process of forming diazonium compounds is called "diazotization". The most important method for the preparation of diazonium salts is treatment of aromatic amines such as aniline with nitrous acid (HNO_2) or Sodium nitrite ($NaNO_2$). Usually the nitrous acid is generated in situ (in the same flask) from sodium nitrite and mineral acid. In aqueous solution diazonium salts are unstable at temperatures above +5 °C; the $-N^{2+}$group tends to be lost as N_2 (nitrogen gas). Often, diazonium compounds are not isolated and once prepared, used immediately in further reactions. This approach is illustrated in the preparation of an aryl halide compound.



Scheme 2.2: Nucleophilic aromatic substitution of diazonium salt with small nucleophile

Procedures

Part 1: Preparation of Diazonium salt

1. Weigh about 0.50 g of *p*-nitroaniline, 5 ml of conc. HCl and 10 ml of water in 250 ml beaker, stir on an ice bath (< 5 °C)

2. Add solution of 0.5 g $NaNO_2$/ 2 ml water, stir for 5 minutes (Carefully! the solution temperature must be below 5 °C).

Part 2: Synthesis of p-iodonitrobenzene

1. Slowly drop solution of 1.0 g KI in 4 ml water into Diazonium salt from part 1. Continuously stir the solution until no bubble appear (Carefully! the solution temperature Must be below 5 °C).

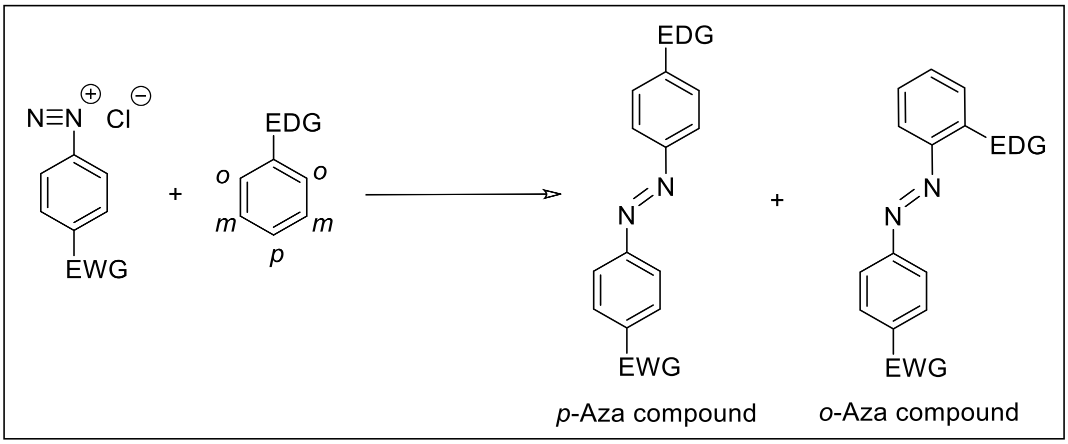
2. Collect the crude of *p*-iodonitrobenzene with vacuum filtration.

Part: 3 Crystallization of p-iodonitrobenzene

1. Dissolve the crude of *p*-iodonitrobenzene with hot Ethanol (EtOH) on hot plate. until the solid completely dissolves.
2. Remove from the hot plate. Allowing the hot mixture to slowly cool to room temperature and cooling further in an ice bath. Crystals will appear. If not, remove the solvent with hot plate.
3. Collect *p*-iodonitrobenzene crystal with vacuum filtration. Record actual weight. What was your percentage yield of *p*-iodonitrobenzene?
4. Explain, what is your major products. Why?

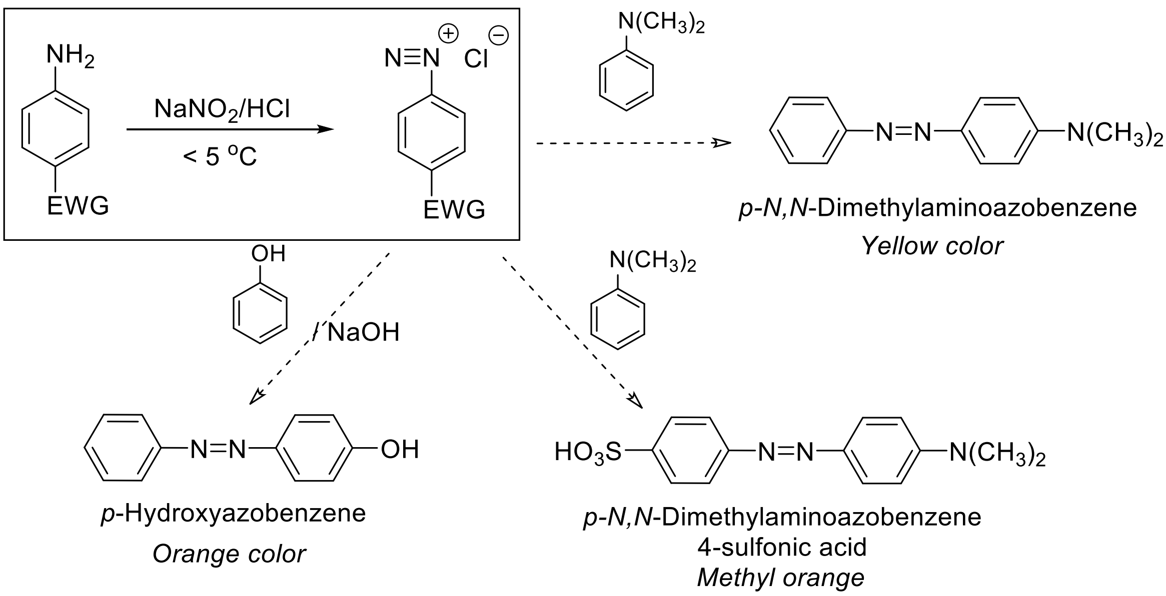
Experiment 3:
Diazonium Salt coupling with β-naphthol: Diazo coupling

An azo coupling is an organic reaction between a diazonium compound and another aromatic compound that produces an azo compound. In this electrophilic aromatic substitution reaction, the aryl diazonium cation is the electrophile and the activated aromatic is a nucleophile. The coupling product results from reaction of the terminal diazonium nitrogen atom at the para- or ortho-position to the activated aromatic.



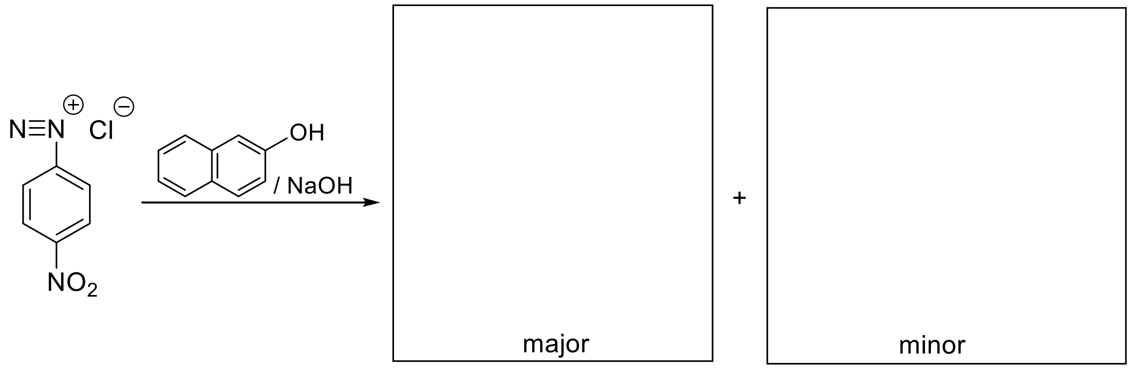
Scheme 3.1: Diazo coupling with bulky nucleophile

Products of diazonium coupling reactions are normally highly conjugated, colored compounds and dye. Important azo dyes include methyl red and pigment red. As shown in scheme 3.2.



Scheme 3.2: Diazo coupling with bulky nucleophile

One example is the synthesis of the dye. Para red can produce from aniline and β-naphthol couples with phenyl diazonium electrophile to produce an intense orange-red dye.



Scheme 3.3: Diazo coupling between Diazonium ion and β-naphthol

Procedures

Part 1: Preparation of Diazonium salt

1. Weigh about 0.50 g of *p*-nitroaniline, 5 ml of conc. HCl and 10 ml of water in 250 ml beaker, stir on an ice bath (< 5 °C)
2. Add solution of 0.5 g $NaNO_2$/ 2 ml water. Carefully the solution temperature must below 10 °C

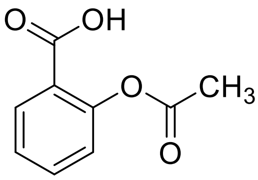
Part 2: Syntheses of para red

1. Slowly drop solution of 0.2 g of β-naphthol in 4 ml of 10% NaOH in to Diazonium salt. Continuous stirring the solution until no bubble appear. Carefully the solution temperature must below 10 °C and stir for 10 minutes.
2. Add dilute HCl till the solution turn to acidity.
3. Collect the para red with vacuum filtration and dried with oven. Record actual weight. What was your percentage yield?
4. Explain and complete Scheme 3.3, what the major and minor of your products. Why?

Experiment 4:

Synthesis of carboxylic derivative; Aspirin

Aspirin (acetylsalicylic acid) is a synthetic organic derived from salicylic acid. Salicylic acid is a natural product found in the bark of the willow tree and was used by the ancient Greeks and Native Americans, among others, to counter fever and pain. However, salicylic acid is bitter and irritates the stomach.



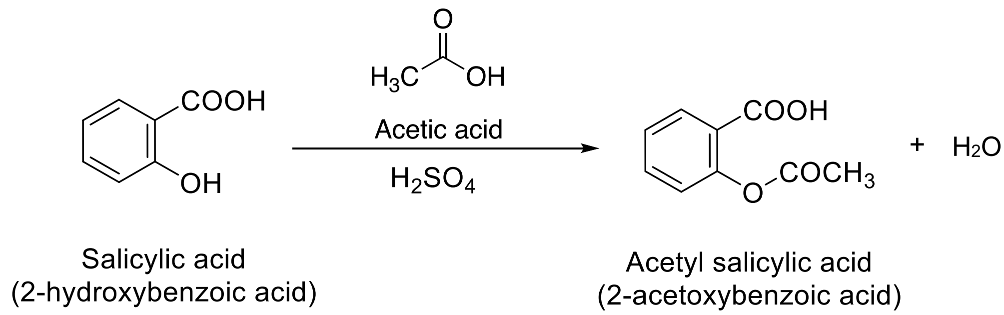
Aspirin
(2-acetoxybenzoic acid)

Scheme 4.1: aspirin structure and pills

A German chemist named Felix Hoffman is credited with being the first to synthesize aspirin in 1897. Hoffman's father had severe arthritis but could not tolerate salicylic acid he was taking for pain relief. The name given for Hoffman's new compound was A-spirin. Apparently, this comes from acetylation (A-), together with Spirin, part of the name for Meadow-sweet (Spiraea ulmaria), a plant rich in salicylates.

Friedrich Bayer, the employer of Hoffman, patented the name and began marketing the product in 1899. It was a huge success and sales grew rapidly. Bayer's company set up by himself, is generally reckoned to have been the first pharmaceutical company, and the production of aspirin is generally accepted to have laid the foundation of the modern pharmaceutical industry.

In this experiment you will synthesize aspirin (acetylsalicylic acid, $C_9H_8O_4$), purify it, and determine the percent yield. The purity of the product will be confirmed by qualitative analysis and by measuring its melting point range.



Scheme 4.2: synthesis of acetyl salicylic acid

The reaction that is used for the synthesis is shown in Scheme 4.2. In this reaction, an excess of acetic acid ($C_2H_4O_2$) is added to a measured mass of salicylic acid ($C_7H_6O_3$) in the presence of a catalyst, sulfuric acid (H_2SO_4). The mixture is heated to form the acetylsalicylic acid ($C_9H_8O_4$) and water. After the reaction takes place, water is added to stop the reaction and cause the product to crystallize. The aspirin is then collected, purified by recrystallization, and its melting temperature measured.

<u>Procedure</u>

Synthesis of Aspirin

1. Staring Materials (1.00 g) of salicylic acid and 7.0 mL of acetic acid from graduated cylinder in 125 mL Erlenmeyer flask. And 10 drops of sulfuric acid (catalyst) to the flask.

......**Be sure** to do this in the hood and wearing your goggles......

......**Don't let** the acetic acid contact your skin and don't get the vapors in your eyes...

2. Reflux the mixture for 45 minutes.

3. Slowly add 20 mL of distilled water to the flask. Swirl the flask to mix the contents.

4. Place it in an ice bath and cool until the crystallization of the aspirin appears complete. If crystals do not appear, you can scratch the walls of the flask with a stirring rod to induce crystallization.

5. Collect the precipitate by Buchner funnel on a vacuum filtration. Wash the filtrate with cool distilled water.

6. Transfer the precipitate into an Erlenmeyer flask. Recrystallizing the product using ethanol. “Dissolve at hot but non-dissolve at cool”

7. Collect the crystal with vacuum filtration. Allow the solid to dry completely

8. Determine weight and %yield of the product.

Explanations!

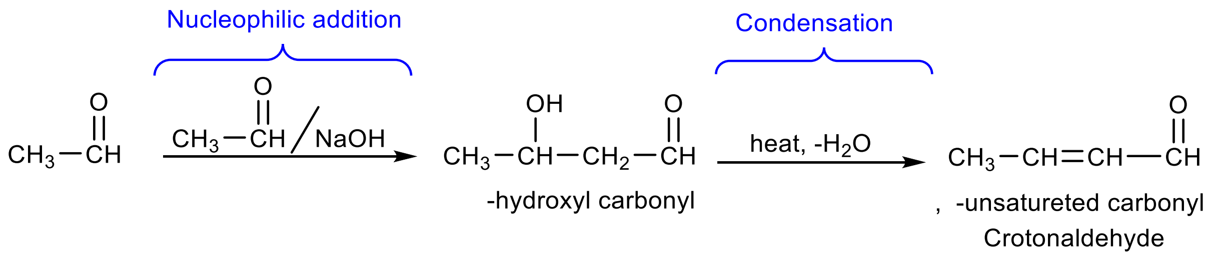
1. Propose the mechanism of the products?

2. What is the possible products? (3 structures)

3. What is the acetyl derivative can use to prepare aspirin?

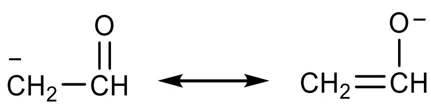
Experiment 5:
The Aldol Condensation

Aldol condensation combines both Nucleophilic addition and Condensation reaction. It is a very useful synthetic reaction for generating new carbon-carbon bond and double bond. In an Aldol reaction, it connects between alpha carbon of one molecule and the carbon of carbonyl of the second molecule. This forms a beta hydroxyl carbonyl compound. Sometime it is followed by dehydration to get alpha-beta unsaturated carbonyl compound (Aldol products).



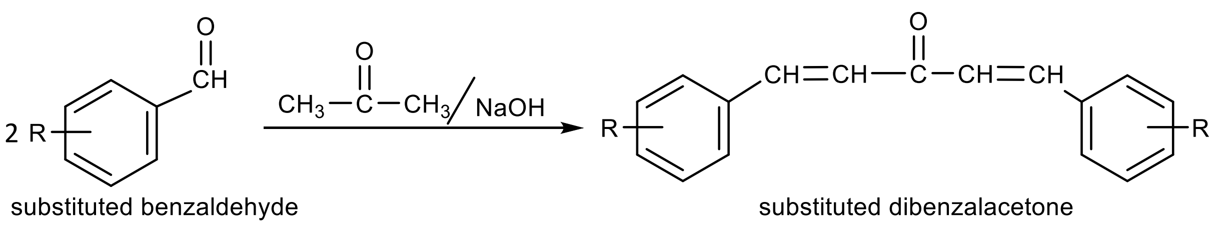
Scheme 5.1: The aldol condensation reaction of acetaldehyde

Mechanistically, the aldol condensation reaction is a simple nucleophilic addition reaction between an enolate ion and a carbonyl group. The enolate is formed when the base (NaOH) present abstracts an α-Hydrogen of a carbonyl one. This Hydrogen is particularly acidic photon because of the resonance stabilization of the resulting enolate. This enolate nucleophilically attacks carbonyl groupings in the typical fashion, forming the aldol product.



Scheme 5.2: The resonance form of enolate anion

In this experiment, we will synthesize the substitute dibenzalacetone from crossed Aldol condensation of acetone and two molecules of a substituted benzaldehyde.



Scheme 5.3: The aldol condensation reaction of substituted benzaldehyde

Procedure

Synthesis of Substituted dibenzalacetone

1. Place 1.4 mL (1.0 eq., 1.458 g) of Benzaldehyde and 0.5 mL (0.5 eq., 0.4 g) of acetone and 10 mL of ethyl alcohol in a 50 mL Erlenmeyer flask
2. Stir the mixture for 3 minutes in an ice water bath.
3. Slowly add 0.5 g of sodium hydroxide (NaOH) in 2 mL of water. Stir for 5 minutes.
4. Remove the ice water bath. Stir the mixture at room temperature for another 10 minutes. The reaction mixture should at first be clear and homogeneous, but should shortly become milky with solid particles of the product.
5. Add the ice and stir until the precipitation occurs.
6. Collect the precipitate by vacuum filtration.
7. Wash the filtrate with 150 mL of distilled water to remove the basicity.
8. Transfer the precipitate into an Erlenmeyer flask.
9. Recrystallize the product using ethyl alcohol.

   "dissolve at hot but non-dissolve at cool"
10. Collect the crystal with vacuum filtration. Allow the solid to dry completely
11. Determine weight and %yield of the product.
12. Determine the melting point.

Explanations!

1. What is the configuration (cis-cis or trans –tran or cis-trans) of the product?

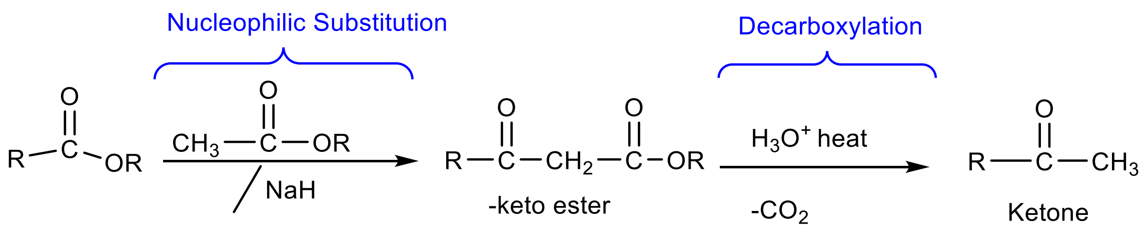
   "The trans-tran-form melts at 110-111 °C;

   The cis-trans-form melts at 60 °C

   The cis-cis-form is an oil"
2. What is the mechanism of the product?
3. Draw the structure of your product in stable-form.

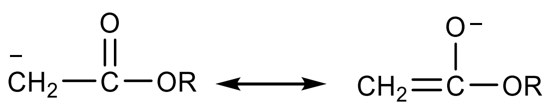
Experiment 6:
The Claisen Condensation

One of the important enolate condensation reactions is the Claisen condensation, which involves reaction of an enolate with the carbonyl compound of an ester. The enolate is generated by removal of a slightly acidic hydrogen from the a-carbon of a ketone, nitrile or ester using a relatively strong base. The enolate then attacks the carbonyl carbon of an ester to yield an intermediate which undergoes rapid loss of the alkoxide leaving group to yield a b-dicarbonyl compound. As usual, all steps in the sequence are completely reversible; the reaction is driven to the product side by deprotonation of the relatively acidic product by the alkoxide ion produced in the condensation. The final product is therefore a new enolate, which can be extracted from the reaction mixture by washing with water, in which it is soluble because it is a salt. It is finally liberated by acidifying the aqueous extract.



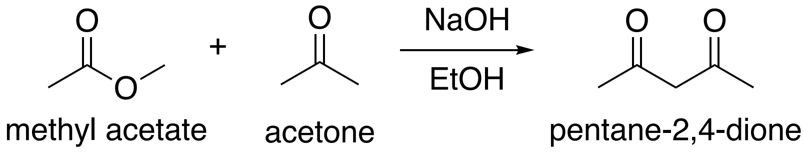
Scheme 5.1: The Claisen condensation reaction of ester

Mechanistically, Claisen condensation reaction is a simple nucleophilic addition reaction between an enolate ion and a carbonyl group. The enolate is formed when the base (NaH) present abstracts an α-Hydrogen of a carbonyl one. This Hydrogen is particularly acidic photon because of the resonance stabilization of the resulting enolate. This enolate nucleophilically attacks carbonyl group in the typical fashion, forming the Claisen product.



Scheme 5.2: The resonance form of enolate anion

In this experiment, we will synthesize the β-diketone from Claisen condensation of methyl acetate and acetone in the presence of a strong base.



Scheme 5.3: The Claisen condensation reaction

Procedure

Synthesis of Substituted dibenzalacetone

1. Place 1.0 mL (1.0 eq., 0.932 g) of methyl acetate, 1.0 mL (1.1 eq., 0.785 g) of acetone and 10 mL of ethyl alcohol in a 50 mL beaker.

2. Add 0.5 g of sodium hydroxide (NaOH). Stir at room temperature for 10 minutes. The reaction is exothermic and will turn from clear liquid to white slurry.

4. Stir the mixture on hot plate under mild heating for another 10 minutes.

5. Cool the mixture to room temperature, add water and stir.

6. Extract the product with 10 mL ethyl acetate for 2 times.

7. Collect the organic phase, dry with anhydrous $NaSO_4$.

8. Transfer the solution into a weighted-beaker.

9. Remove the solvent on hot plate. (Don’t forget to add boiling chips!)

10. Determine weight and %yield of the product.

11. Check TLC of the product.

Explanations!

1. What is the mechanism of this reaction?

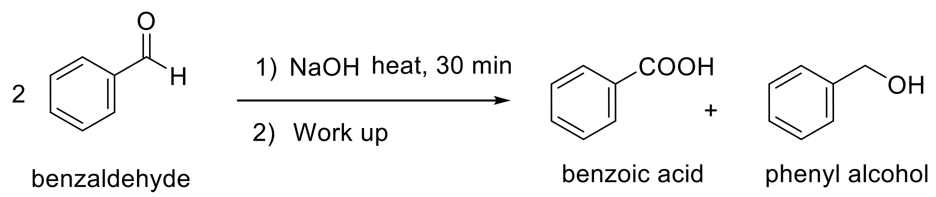
2. Draw the structure of your product in stable-form.

Experiment 7:

Cannizzaro Reaction of benzaldehyde

As a general rule, nucleophilic addition reactions are characteristic only of aldehydes and ketones, not of carboxylic acid derivatives. The reason for the difference of is structural; the tetrahedral intermediate produced by addition of a nucleophile to a carboxylic acid derivative can eliminate a leaving group, leading to a net nucleophilic acyl substitution reaction. The tetrahedral intermediate produced by addition of a nucleophile to an aldehyde or ketone, however, has only alkyl or hydrogen substituents and thus can't usually expel a leaving group. One exception to this rule, however, is the Cannizaro reaction, discovered in 1853.

The Cannizzaro reaction takes place by nucleophilic addition of OH- to an nonenolizable aldehyde (bearing no α–H) to give a tetrahedral intermediate, which expels hydride ion as a leaving group and is thereby oxidized. A second aldehyde molecule accepts the hydride ion in another nucleophilic addition step and is thereby reduced



Scheme 7.1: Cannizzaro Reaction of benzaldehyde

The Cannizzaro reaction is that of aldehydes that do not contain alpha hydrogens to give carboxylic acids and alcohols (alpha hydrogens cause an Aldol reaction to take place). This occurs in the presence of a strong base. Benzaldehyde, which does not contain alpha hydrogens, was used for this reaction.

The reflux technique will be used in order to allow for a complete reaction between all the components. On completion of the reaction, the extraction technique will enable the separation of benzoic acid and benzyl alcohol. This will be achieved by the ionization of

Benzoic acid which will separate from the organic benzyl alcohol. Reacidification will reform the Benzoic acid.

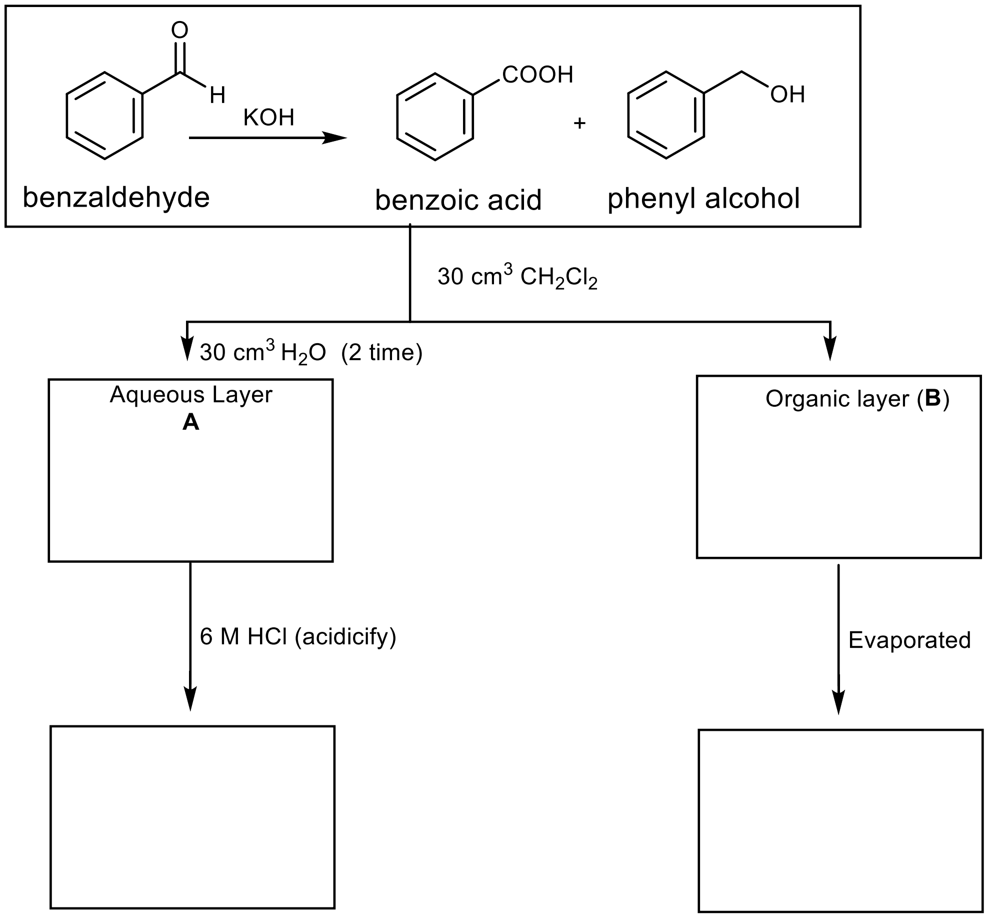
Procedure

1. Place about 3.0 ml of benzaldehyde, 15 ml of methanol and 2 ml of aqueous 10 M KOH in a 100 ml round bottom flask; add a couple of boiling chips finally.
2. Reflux the reaction mixture and heat the reaction mixture to the boiling point of methanol for 30 minute. Allow the mixture to cool down and place in an ice bath.
3. Transfer the mixture carefully to a separator funnel. Addition 30 ml of CH_2Cl_2 and Extraction two time with 30 ml of water
4. The aqueous phase was collected in Flask **A**. and the aqueous phase was work up with HCl to appear crystal.
5. Collect the crystal with vacuum filtration. Allow the solid to dry completely, Determine weight and %yield of the product **A**.

Organic Phase Work-up:

1. The CH_2Cl_2 phase was collected in Flask **B**. it was dries over by anhydrous MgSO4. Add enough drying agent so, the solution appears clear as opposed as cloudy.
2. Rinse the CH_2Cl_2 to beaker (weight).
3. In the hood, evaporate the CH_2Cl_2 by using a hot plate.
4. Determine the yield of the oily residue of the product **B**.

--------Then take the product A and B ot IR spectroscopy---------



Scheme 7.2 the synthesis and extraction of cannizzaro reaction

Experiment 8:

Identification of Cannizzaro Reaction Product by FT-IR technique

Infrared spectroscopy is certainly one of the most important analytical technique available to today's scientists. Infrared spectroscopy is a technique based on the vibrations of the atoms of a molecule. An infrared spectrum is commonly obtained by passing infrared radiation through a sample and determining what fraction of the incident radiation is absorbed at a particular energy. The energy at which any peak in an absorption spectrum appears corresponds to the frequency of a vibration of a part of a sample molecule. In this introductory chapter, the basic ideas and definitions associated with infrared spectroscopy will be described. The vibrations of molecules will be looked at here, as these are crucial to the interpretation of infrared spectra.

This equation may be modified so that direct use of the wavenumber values for bond vibrational frequencies can be made, namely:

$$\bar{\nu} = \frac{1}{2\pi c}\sqrt{\frac{K}{\mu}}$$

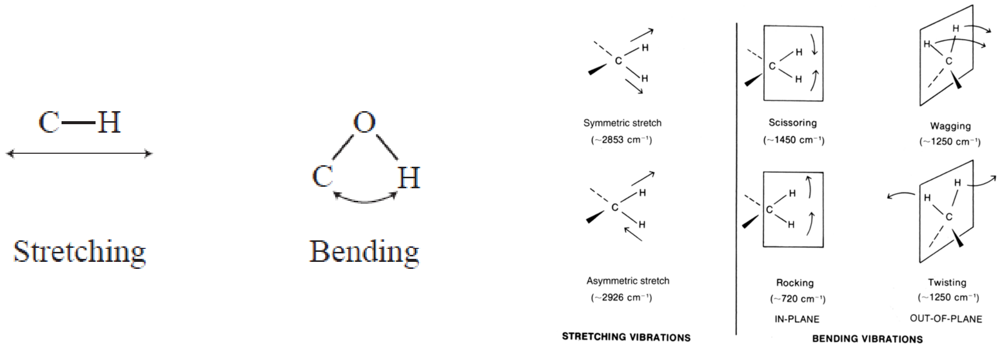
$\bar{\nu}$ = frequency in cm^{-1}

c = velocity of light = 3×10^{10} cm/sec

K = force constant in dynes/cm

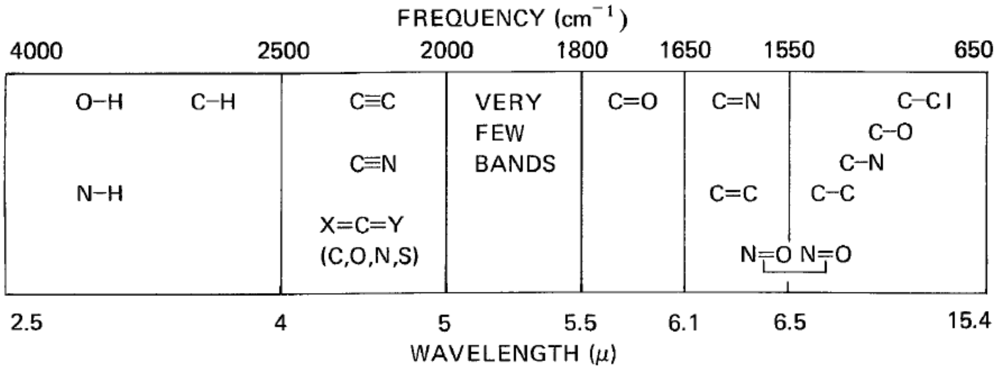
$\mu = \frac{m_1 m_2}{m_1 + m_2}$, masses of atoms in grams

The two primary modes of vibration are stretching and bending Stretching modes are typically of higher energy than bending modes Stretching modes are often divided into two a symmetric and asymmetric stretch; the asymmetric stretch is usually of higher energy



Scheme 8.1 the mode of vibration in organic compounds

A second and more important use of the infrared spectrum is Functional Groups of a molecule. The absorptions of each type of bond (N—H, C—H, O—H, C—X, C—O, C═O, C—C, C═C, C≡C, C≡N, and so on) are regularly found only in certain small portions of the vibrational infrared region. A small range of absorption can be defined for each type of bond. The same type of range applies to each type of bond. Scheme 8.1 illustrates schematically how these are spread out over the vibrational infrared. Try to fix this general scheme in your mind for future convenience.



Scheme 8.2 The approximate regions where various common types of bonds absorb (stretching vibrations only; bending, twisting, and other types of bond vibrations have been omitted for clarity).

In general, the frequencies range of vibration stretching of single, double and triple bonds between two atoms are following bonds

C—H 2900-3300 cm^{-1} $^{-1}$

C—C 1200 cm^{-1} C═C 1650 cm^{-1} C≡C 2150 cm^{-1}

C—O 1100 cm^{-1} C═O 1800 cm^{-1} C≡N 2250 cm^{-1}

O—H 3200-3600 cm^{-1}

N—H 3300 cm^{-1}

The Canizzaro reaction for previous experiment benzoic acid and phenyl alcohol were produced. The infrared (IR) absorption occurs from the stretching and bending of the covalent bonds in molecules to be accompanied by IR absorption a stretch or bend must change the dipole moment of the molecule

Sample Preparation for IR Work

Three major methods of sample preparation

1. Sample is mixed with a mulling agent such mineral oil and pressed between plates made of Sodium chloride is used because it has no IR absorptions; glass or plastic plates would have IR absorptions of their own Sodium chloride plates are good from 4000 to 650 cm^{-1}; below 650 cm^{-1} they begin to absorb

Potassium bromide plates can be used in place of sodium chloride and are transparent to 400 cm^{-1}, but they are more expensive.

Downside of this method is absorptions due to the mineral oil

2. Sample is mixed with solid potassium bromide and pressed into a pellet under high pressureNo absorptions from mulling agent. Only works for solids

3. Sample is dissolved in carbon tetrachloride and pressed between salt plates

Downside of this method is absorptions due to the CCl_4

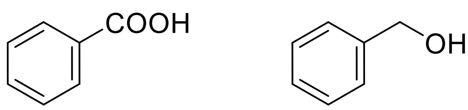
<u>**Procedure**</u>

Sample Preparation

1. The product **A** and **B** were prepared for FT-IR spectrometer.

2. Try to assign at least three of the most prominent bands in the spectrum. Are they consistent with the Functional Group you believe is present in your sample?

3. The laboratory work involves identification of an unknown by recording its infrared spectrum, investigating the major absorption bands, and comparing the spectrum product **A** and **B** with spectra of benzoic acid and phenyl alcohol.



benzoic acid phenyl alcohol

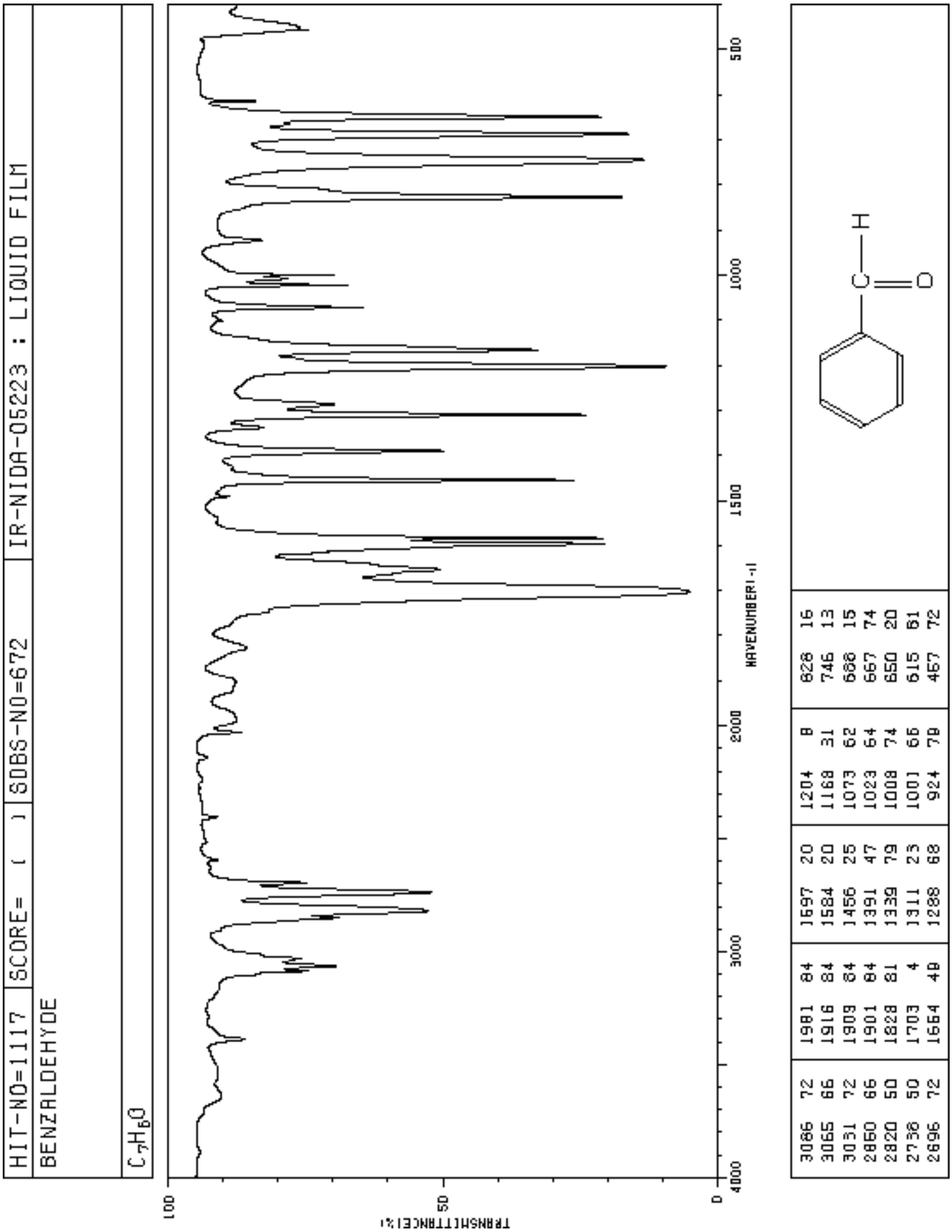
Explanations!

1. Give the mechanism of the reaction and complete Scheme 7.1

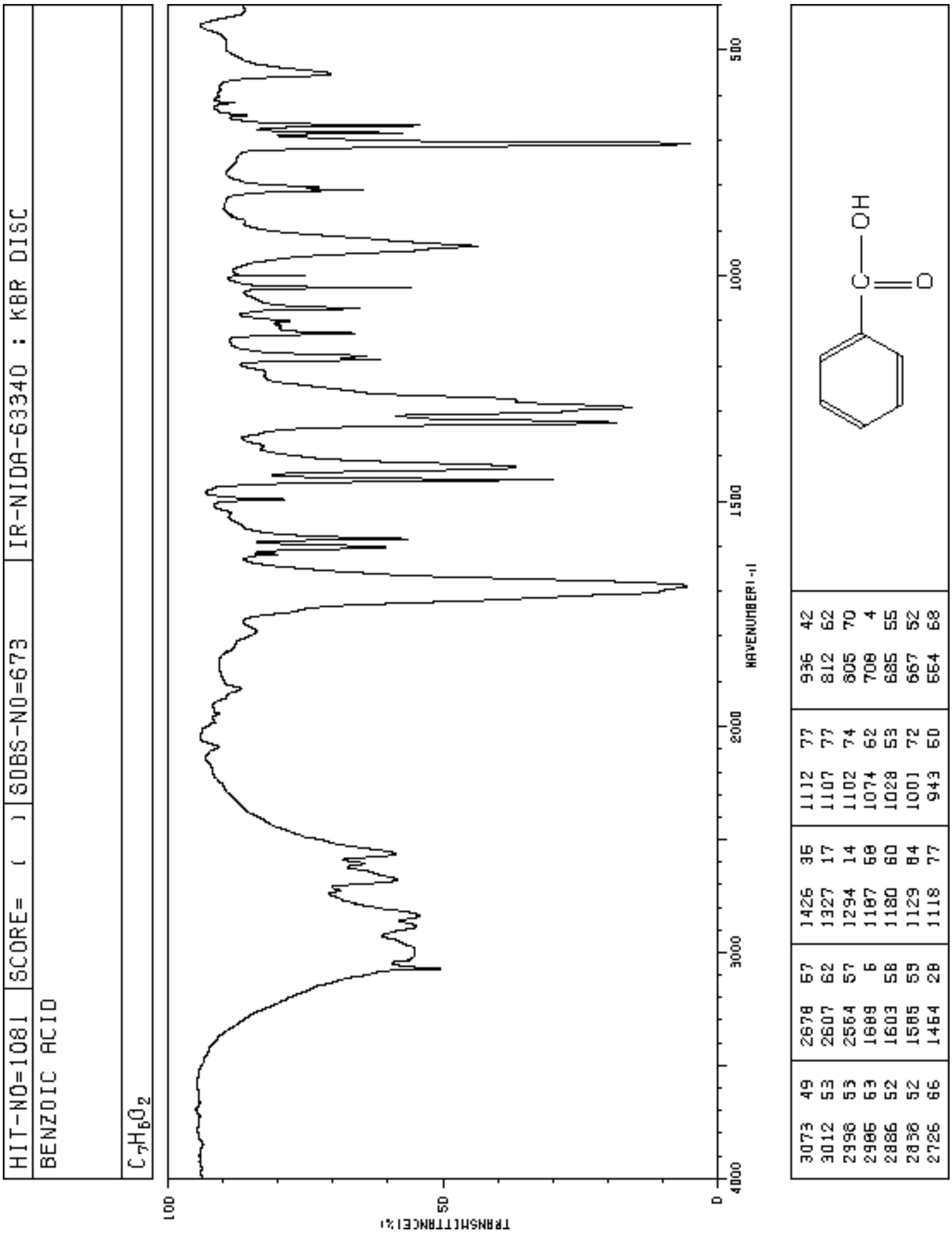
2. How would you isolate the alcohol from the organic layer that contains also the portion of unreacted aldehyde?

3. Calculate the mass of unreacted benzaldehyde.

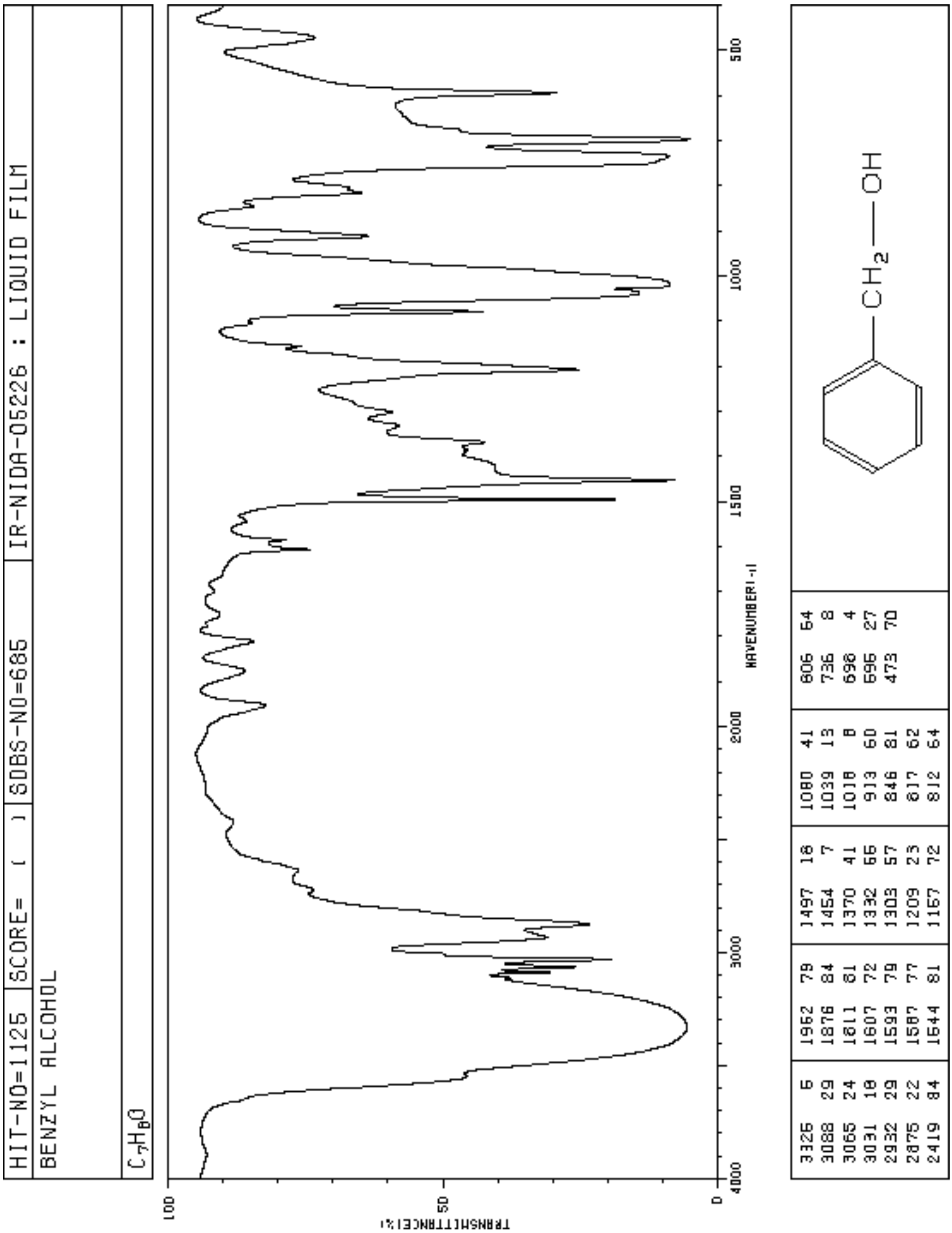
IR Spectrum - Benzaldehyde



IR Spectrum – Benzoic acid



IR Spectrum – Benzyl alcohol



Experiment 9:

Carbohydrate: Isolation of lactose from Milk

Milk is a food of exceptional interest. Not only is milk an excellent food for the very young, but humans have also adapted milk, specifically cow's milk, as a food substance for persons of all ages. Many specialized milk products like cheese, yogurt, butter, and ice cream are staples of our diet. Milk is probably the most nutritionally-complete food that can be found in nature. This property is important for milk, since it is the only food young mammals consume in the nutritionally significant weeks following birth. Whole milk contains vitamins (principally thiamine, riboflavin, pantothenic acid, and vitamins A, D, and K), minerals (calcium, potassium, sodium, phosphorus, and trace metals), proteins (which include all the essential amino acids), carbohydrates (chiefly lactose), and lipids (fats).

The only important elements in which milk is seriously deficient are iron and Vitamin C. Infants are usually born with a storage supply of iron large enough to meet their needs for several weeks. Vitamin C is easily secured through an orange juice supplement. The average composition of the milk of each of several mammals is summarized in the accompanying table.

Table 6.1 Average percentage composition of Milk from Various Mammals

	Cow	Human	Goat	Sheep	Horse
Water	87.1	87.4	87.0	82.6	90.6
Protein	3.4	1.4	3.3	5.5	2.0
Fats	3.9	4.0	4.2	6.5	1.1
Carbohydrates	4.9	7.0	4.8	4.5	5.9
Minerals	0.7	0.2	0.7	0.9	0.4

Fats

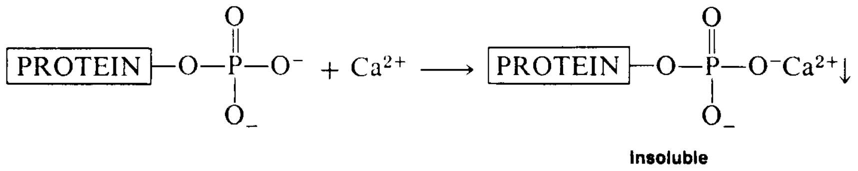
Whole milk is an oil-water type of emulsion, containing about 4% fat dispersed as very small (5-10 microns in diameter) globules. The globules are so small that a drop of milk contains about a million of them. Because the fat in milk is so finely dispersed, it is digested more easily than fat from any other source. The fat emulsion is stabilized to some extent by complex phospholipids and proteins that are adsorbed on the surfaces of the globules. Skimmed milk, except for lacking the fats and vitamins A and D, has approximately the same composition as whole milk. If milk is homogenized, its fatty content will not separate. Milk is homogenized by forcing it through a small hole. This breaks up the fat globules and reduces their size to about 1 to 2 microns in diameter.

Proteins

Proteins may be classified broadly in two general categories: fibrous and globular. Globular proteins are those that tend to fold back on themselves into compact units that approach nearly spheroidal shapes. These types of proteins do not form intermolecular interactions between protein units (H bonds and so on) as fibrous proteins do, and they are more easily solubilized as colloidal suspensions. There are three kinds of proteins in milk: caseins, lactalbumins, and lactoglobulins. All are globular.

Casein is a phosphoprotein, which has phosphate groups are attached to some of the amino acid side- chains. These are attached mainly to the hydroxyl groups of the serine and threonine moieties. Actually, casein is a mixture of at least three similar proteins, which differ primarily in molecular weight and amount of phosphorus they contain (number of phosphate groups). Casein exists in milk as the calcium salt, calcium caseinate. This salt has a complex structure. It is composed of α, β and κ-caseins which form a micelle, or a solubilized unit. Neither the α nor the β casein is soluble in milk, singly or in combination. If κ casein is added to either one, or to a combination of the two, however, the result is a casein complex that is soluble owing to the formation of the micelle.

A structure proposed for the casein micelle is shown on the following page. The ***K*** casein is thought to stabilize the micelle. Since both α and β casein are phosphoproteins, they are precipitated by calcium ions.



Calcium caseinate has its isoelectric (neutrality) point at pH 4.6. Therefore, it is insoluble in solutions of pH less than 4.6. The pH of milk is about 6.6; therefore casein has a negative charge at this pH and is solubilized as a salt. If acid is added to milk, the negative charges on the outer surface of the micelle are neutralized (the phosphate groups are protonated) and the neutral protein precipitates:

$$Ca^{2+}\text{Caseinate} + 2HCl \longrightarrow \text{Casein} + CaCl_2$$

The calcium ions remain in solution. When milk sours, lactic acid is produced by bacterial action (see below), and the consequent lowering of the pH causes the same clotting reaction. The isolation of casein from milk will be carried out in this experiment.

The casein in milk can also be clotted by the action of an enzyme called rennin. Rennin is found in the fourth stomach of young calves. However, both the nature of the clot and the mechanism of clotting differ when rennin is used. The clot formed using rennin, calcium paracaseinate, contains calcium.

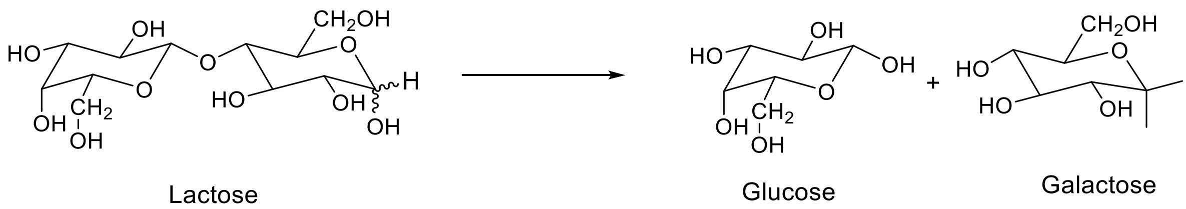
$$Ca^{2+}\text{Caseinate} + \text{rennin} \longrightarrow Ca^{2+}\text{Paracaseinate}\downarrow + \text{a small peptide}$$

Rennin is a hydrolytic enzyme (peptidase) and acts specifically to cleave peptide bonds between phenylalanine and methionine residues. It attacks the **K** casein, breaking the peptide chain so as to release a small segment of it. This destroys the water-solubilizing surface of the **K** casein, which protects the inner α and β caseins and causes the entire micelle to precipitate as calcium paracaseinate. Milk can be decalcified by treatment with oxalate ion, which forms an insoluble calcium salt. If the calcium ions are removed from milk, a clot will not be formed when the milk is treated with rennin.

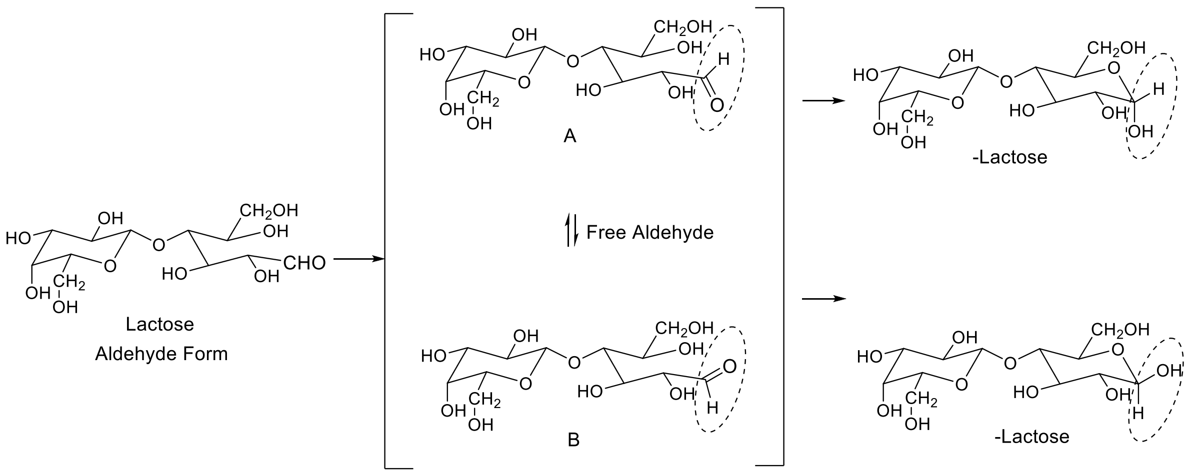
The clot, or curd, formed by the action of rennin is sold commercially as cottage cheese. The liquid remaining is called the whey. The curd can also be used in producing various types of cheese. It is washed, pressed to remove any excess whey, and chopped. After this treatment, it is melted, hardened and ground. The ground curd is then salted, pressed into molds, and set aside to age.

CARBOHYDRATES

When the fats and the proteins have been removed from milk, the carbohydrates remain, as they are soluble in aqueous solution. The main carbohydrate in milk is lactose. Lactose, a disaccharide, is the only carbohydrate that mammals synthesize. It is synthesized in the mammary glands. Hydrolyzed, it yields one molecule of D-glucose and one of D-galactose.



Lactose is an example of a disaccharide. It is made up of two sugar molecules: galactose and glucose. In the following structures, the galactose portion is on the left and glucose is on the right. Galactose is bonded through an acetal linkage to glucose



Notice that the glucose portion can exist in one of two isomeric hemiacetal structures: α-lactose and β- lactose. Glucose can also exist in a free aldehyde form. This aldehyde form (open form) is an intermediate in the equilibration (interconversion) of α and β-lactose. Very little of this free aldehyde form exists in the equilibrium mixture. The isomeric α and β-lactose are diastereomers because they differ in the configuration at one carbon atom, called the anomeric carbon atom.

The sugar α -lactose is easily obtainable by crystallization from a water-ethanol mixture at room temperature. On the other hand, β-lactose must be obtained by a more difficult process, which involves crystallization from a concentrated solution of lactose at temperatures about 93.5°C . In the present experiment, α-lactose is isolated by the simpler experimental procedure indicated above. α- Lactose undergoes numerous interesting reactions. First, α-lactose interconverts, via the free aldehyde form, to a large extent, to the β-isomer in aqueous solution. This causes a change in the rotation of polarized light from $+92.60^{\circ}$ to $+52.30^{\circ}$ with increasing time. The process that causes the change in optical rotation with time is called **mutarotation**.

Procedures

Part I: Isolation of Lactose

1. Weigh out 5.00 g of non-fat powdered milk. Dissolve the milk in 100 mL of warm water in a 250mL beaker.

2. Heat the milk solution to 40 °C.

3. Slowly drop 6 mL of 10% Acetic Acid to precipitate out the Casein, and stir the mixture slowly and briefly. Avoid adding excess Acetic Acid. Work the Casein into a mass and remove it with a stirring rod or spoon.

4. Decant the solution and Filter with cotton wool.

5. Add 1.25 g of powdered Calcium Carbonate. Stir the mixture thoroughly

. 6. Heat the mixture almost to boiling for about 10 minutes; stirring continuously. This should precipitate the remaining proteins.

7. Filter the hot mixture with cotton wool, collecting the filtrate in a 250mL beaker.

8. Stir the filtrate continuously while boiling it down to about 7.5 mL, and then add 50 mL of 95% Ethanol.

9. Filter the warm Ethanol solution with vacuum, collecting the filtrate in a 125mL Erlenmeyer flask. Stopper the flask and place it in your lab drawer until the next lab period.

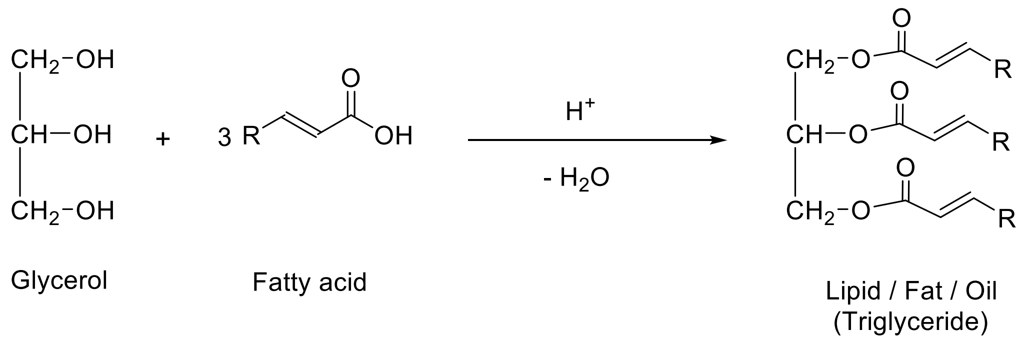
10. Filter off the crystals of Lactose. Allow them to dry. Weigh the crystals.

11. Compare your percentage recovery of Lactose with the percentage carbohydrate as reported on the nutritional label of the powdered milk you used.

Experiment 10:

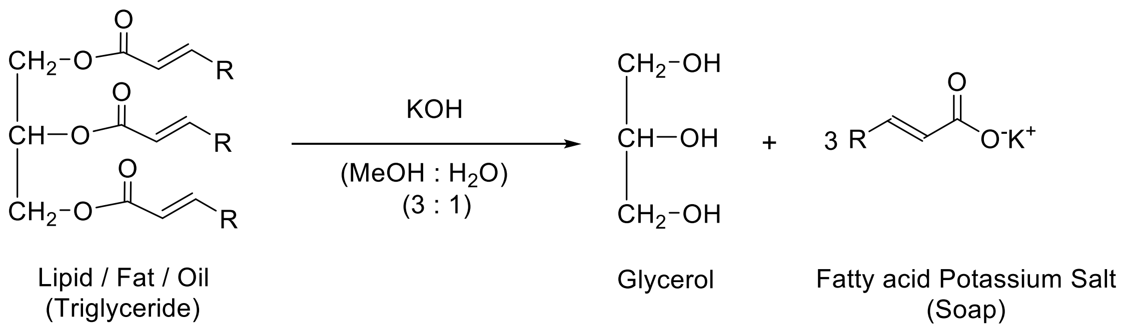
Lipid; Biodiesel; Saponification

Fats and oils are the principle stored forms of energy in many organisms. They are highly reduced compounds and are derivatives of fatty acids. Fatty acids are carboxylic acids with hydrocarbon chains of 4 to 36 carbons, they can be saturated or unsaturated. The simplest lipids constructed from fatty acids are triacylglycerols or triglycerides. Triacylglycerols are composed of three fatty acids each in ester linkage with a single glycerol. Since the polar hydroxyls of glycerol and the polar carboxylates of the fatty acids are bound in ester linkages, triacyl glycerols are non-polar, hydrophobic molecules, which are insoluble in water.



Saponification is the hydrolysis of fats or oils under basic conditions to afford glycerol and the salt of the corresponding fatty acid. Saponification literally means "soap making". It is important to the industrial user to know the amount of free fatty acid present, since this determines in large measure the refining loss. The amount of free fatty acid is estimated by determining the quantity of alkali that must be added to the fat to render it neutral. This is done by warming a known amount of the fat with strong aqueous caustic soda solution, which converts the free fatty acid into soap. This soap is then removed and the amount of fat remaining is then determined. The loss is estimated by subtracting this amount from the amount of fat originally taken for the test.

Saponification reaction



<u>**The saponification number is** the number of milligrams of potassium hydroxide required to neutralize the fatty acids resulting from the complete hydrolysis of 1g of fat.</u>

Saponification number = mg of KOH consumes by 1g of fat

It gives information concerning the character of the fatty acids of the fat- the longer the carbon chain, the less acid is liberated per gram of fat hydrolyzed. It is also considered as a measure of the average molecular weight (or chain length) of all the fatty acids present. The long chain fatty acids found in fats have low saponification value because they have a relatively fewer number of carboxylic functional groups per unit mass of the fat and therefore high molecular weight. The average molecular weight (MW) of fat can calculate from the saponification number.

The average molecular weight of fat = 3 x Saponification number

Table 10.1: the saponification number of lipids

Lipids	Saponification number
Coconut oil	248-285
Palm oil	190-209
Olive oil	190-195
Soy oil	189-194

Procedures

Saponification and titration

1. Weigh 4.0 g of fat (Sample X) in 250 mL of round-bottom **flask A**.
   And blank (not thing) in 250 mL of round-bottom **flask B**.
2. Slowly add 50.0 mL of 0.5N ethanolic KOH to both the flasks
   (Weigh 1.4 g of KOH dissolve in EtOH : H_2O/ 3 : 1) and stir for 5 minutes.
3. Reflux and stir the solution for 30 minutes, until the solution no longer has two separate layers.
4. Cool the flasks to room temperature
5. Add phenolphthalein indicator to both the flasks and titrate with 0.5N HCl.
6. Note down the endpoint of test (**flask A**) and blank (**flask B**).
7. Calculate the saponification value using the formula

$$\boxed{\text{Saponification Number (average)} = \frac{56.1\,N\,(x-v)}{W}}$$

N = The concentration of HCl in normality
x = The volume of HCl in litres (blank **Flask B**)
v = The volume of HCl in litres (test **Flask A**)
W = Weight of fat (gram)

8. Compare your result with table 10.1. Explain why?
9. Show calculation of the average molecular weight (MW) of your fat.