Practical Problems

Safety rules

These rules have been accepted by the International Jury of the Chemistry Olympiad. All students are expected to be familiar with these rules before they take part in the Olympiad. A brief instruction will be given on the day preceding the examination.

1. Be attentive to instructions and follow them carefully. Maintain a business-like attitude. Be prepared! Learn the location and the proper use of the fire extinguisher, safety shower, eye and face wash. If you have any questions about the procedures, apparatus or chemicals it is important to ask the instructor.
2. All experiments in which dangerous or obnoxious fumes are produced, must be done in the fume hood. Be sure to stop these reactions as soon as possible.
3. A pair of safety spectacles which conform to the Standards with side shields must be worn at all times in the lab. If you wear contact lenses they must be covered with safety goggles that provide total protection around the eyes. Prescription spectacles with polycarbonate lenses are acceptable provided they include side shields. Eye injuries must be prevented at all costs. The instructor in charge will check all protective equipment to ensure if this is satisfactory before admittance to the examination laboratory. We have asked the teams to bring their own safety glasses, but we will have some in reserve.
4. A long sleeved knee length laboratory coat and shoes that enclose the feet must be worn. Thongs, sandals and open style shoes are prohibited. Long hair (below the collar) must be contained. Please ensure that you bring a lab coat, flat-heeled non-slip shoes and suitable hair restraints.
5. Drinking, eating, smoking or chewing of gum in laboratories in strictly prohibited.
6. All accidents must be reported. In any chemical laboratory there is danger from accidental spillage of chemicals, broken glass or fire. Part of your training in practical chemistry is to learn the procedures that allow safe working conditions. Specific safety precautions relating to particular experiments are detailed in the practical task notes.
7. All chemicals must be treated with respect, some are corrosive, some maybe poisonous and many, especially organic chemicals, are inflammable. When using a chemical for the first time you should always read the safety notes included in the practical task paper.
8. Never taste chemicals, or use pipettes by mouth. Always employ pipette bulbs.
9. Avoid unnecessary contact with all chemicals. In case of any spillage of chemicals on the skin, flush the skin under running cold water from the tap or the shower hose for a minimum of 10 minutes and then seek first aid /medical treatment as appropriate. Organic materials can be absorbed through the skin in these cases flush with water, wash with soap, rinse with water, rinse with ethyl alcohol, wash with soap, and rinse with water. Pat dry. Contaminated clothing should be removed as soon as possible and thoroughly washed.
10. Chemicals in the eye should be flushed immediately with copious amounts of water using the eyewash fountain. Seek first aid/medical treatment as appropriate.
11. Many chemicals are inflammable and no open flames are permitted when such chemicals are in use. You are required to know the location of the nearest fire extinguisher, fire blanket and safety shower. In case of a person's clothing catches fire put him/her on the floor and roll him/her to smother the flames. Use the fire blanket or a laboratory coat if accessible. Do not allow the person to run or stand, even if using a safety shower, to prevent rising flames from reaching the head. Do not use any type of chemical fire extinguisher on a person.
12. If there is a fire in the lab where you working shout 'fire' to alert your neighbours and instructor. A small fire in a test tube can usually be extinguished by covering the container with a watch glass. If the fire cannot be extinguished by one extinguisher or by sand or water, you will be instructed to evacuate.
13. In case of evacuation the following procedures should be maintained, stay calm, extinguish any flames and turn off electrical equipment. Close any open windows and internal doors near you. Walk

quickly (but do not run!) to the nearest exit and leave the building. Make sure that after evacuation your name is on the list of evacuated persons so there's no doubt about people missing.

14. Handle all glassware carefully. Glass is a very hard but brittle material and breaks readily under stress or strain. If cuts or punctures occur it is essential that any particles or splinters of glass in any wound is removed. All cuts must be inspected by the instructor in charge. Report and clean up any breakages of glassware, replacements may be obtained by the instructor in charge.

15. Chemical waste material must not be washed away. No chemicals are to enter the drainage system. Particular care must be taken in the disposal of some reaction residues. Where this is important details will be given in the notes and labelled designated waste residue bottles will be provided.

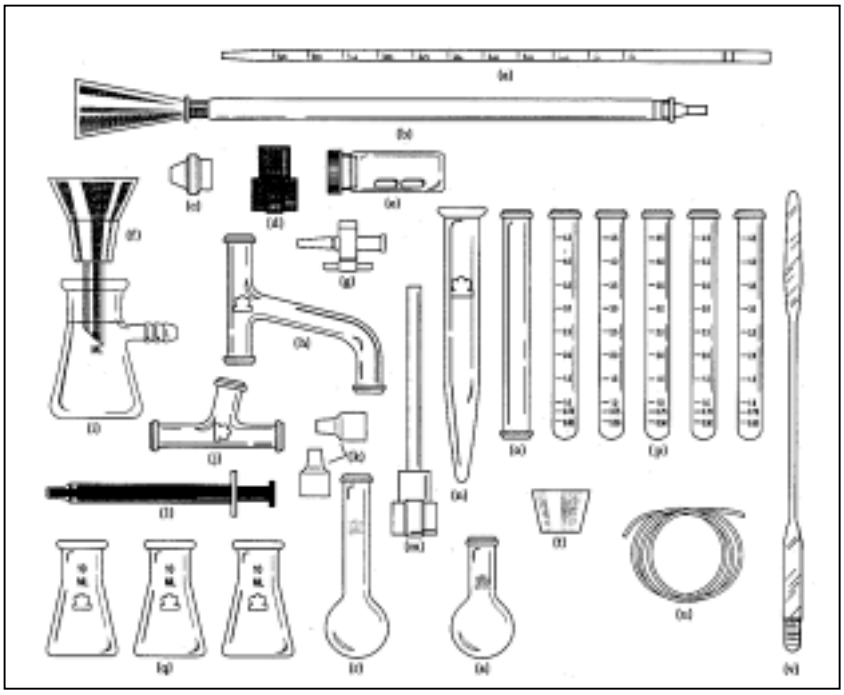
16. Each student is responsible for his/her section of the bench. Any chemicals or water spill must be wiped up immediately. Concentrated acid spills should first be neutralised with sodium bicarbonate and then washed away with plenty of cold water. Your working area must be kept clean at all times. Chemicals spilt on the floor must be washed away immediately and broken glass swept up.

17. Before leaving the laboratory, check that the water, steam, heaters and gas faucets are shut off.

Hints for the practical exam

This year micro scale equipment will be used in the practical exam. Since it is the first times that this equipment will be used in the practical part of an Olympiad, a short introduction is given. The micro scale glasswork is provided in a case, which has the following contents:

(a) Pipette 1 mL
(b) Chromatography column
(c) Thermometer adaptor
(d) Connector
(e) Magnetic stirring bars
(f) Hirsch funnel
(g) One-way stopcock
(h) Distillation head 60 mm
(i) Filter flask 25 mL
(j) Connecting adaptor
(k) Sleeve stopper 8 mm septum
(l) Syringe polyethylene 1 mL
(m) Connector with support rod
(n) Centrifuge Tube 15 mL
(o) Distillation column
(p) Reaction tube 10 x 100 mm
(q) Erlenmeyer flask 10 mL
(r) Long neck flask 5 mL
(s) Short neck flask 5 mL
(t) Filter adapter
(u) Tubing PTFE 1/16"
(v) Spatula



Important notice:

The experiments described in this section of the preparatory problems can also be carried out using any kind of small-size glassware. The working scale will be circa 400 mg. On the day preceding the practical exam a brief demonstration of the use of the micro scale equipment will be given. If so desired a case of the micro scale equipment can be purchased, for details see the website of the IChO34.

During the practical exam open flames will not be used, instead we will have an electrical heating device. We do not intend to use any smelly or dangerous chemicals.

Using this glassware most apparatus can be built up in the same way that you are use to build regular scale apparatus. The connector (d) is used to couple the different glassware. However, be careful not to push the edges of the glass sections against each other. Some examples of the apparatus that can be built with micro scale glasswork are given below.

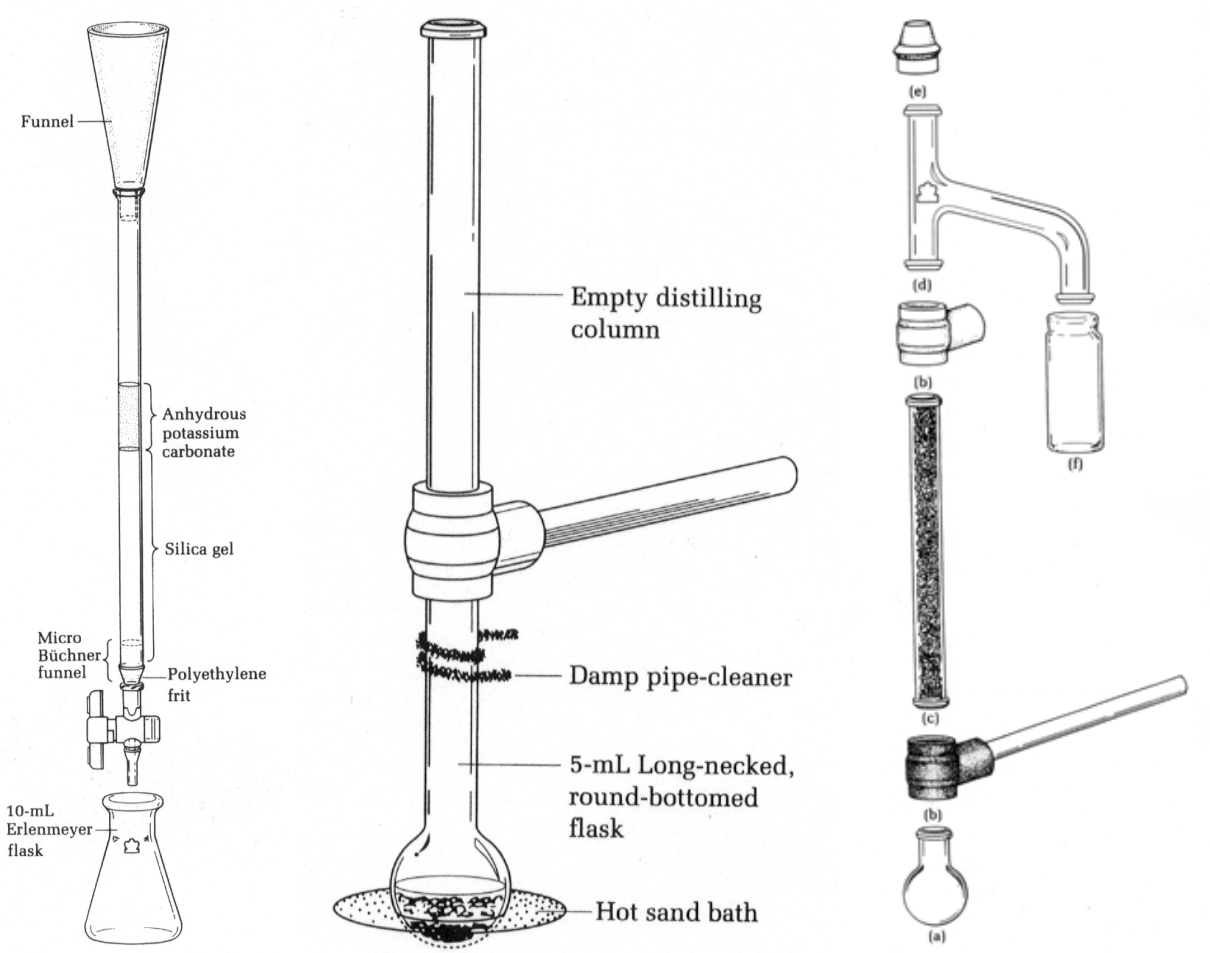


Figure 1
(a) Chromatography column (b) Reflux apparatus (c) Fractional distillation apparatus

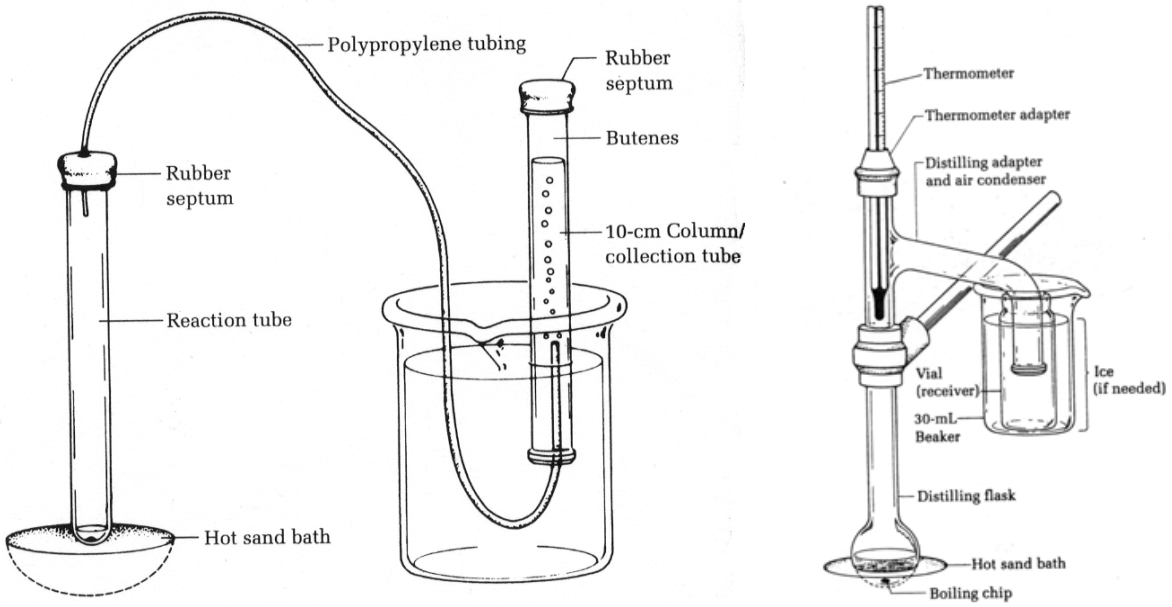
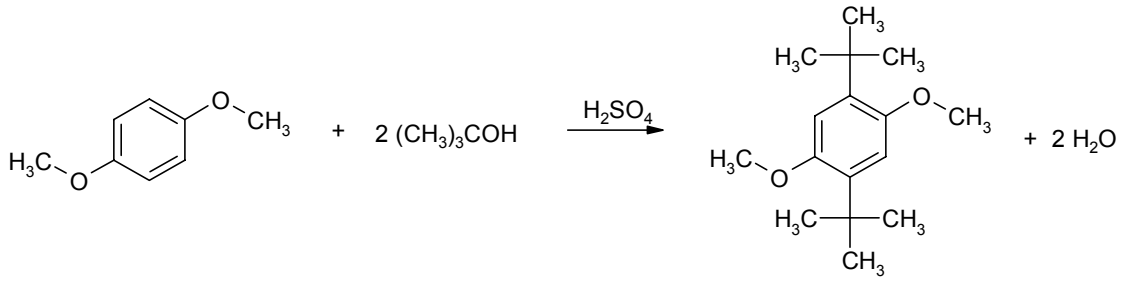


Figure 2
(a) Gas collecting apparatus (b) Distillation apparatus

Problem 24 Experiment

Preparation of 1,4-di-*tert*-butyl-2,5-dimethoxybenzene; an example of a Friedel-Crafts alkylation.

The equation for the di-*tert*-butylation of 1,4-dimethoxybenzene is as follows:



Procedure

A stirred mixture of 1,4-dimethoxybenzene (360 mg) in glacial acetic acid (15 mL) contained in an Erlenmeyer flask of 50 mL is heated gently (water bath) until dissolved. *tert*-Butyl alcohol (0.6 mL) is then added using a pipette and the mixture is cooled in a crushed ice bath, while stirring is continued, and treated dropwise with concentrated sulfuric acid (45 drops). The addition is achieved using a Pasteur pipette and it must be ensured that each drop is mixed thoroughly with the reaction mixture before the next drop is added. The mixture is then removed from the ice bath, allowed to attain room temperature, and then stirred for a further 25 minutes. The mixture is again cooled in an ice-bath (0^{o} C) whereon water (3 drops) is added VERY CAREFULLY with slow stirring. Crystallization of the product begins and is accelerated by the slow, careful addition of ice water (7.5 mL). After ca.10 minutes the crystalline material is collected by filtration using a Hirsch funnel. The crystals are washed twice with water and allowed to dry. The product is recrystallized by dissolving in hot methanol (10mL) and cooling in an ice bath and again collected using a Hirsch funnel. The material is allowed to dry on the air. Determine the weight, calculate the yield and determine the melting point. The purity of the product is determined by thin-layer chromatography (silica gel 60 F254) using hexane as the eluent. The starting material is run on the same TLC plate as a reference. Determine the R_f-value of the product, of the starting material and of any contaminant in the product if present.

Record the following data:
1. The weight of the product.
2. The yield of product in percentage of the theoretical yield (show your calculation).
3. The appearance of the crystals and their color.
4. The m.p.
5. Copy your TLC plate on the data sheet + the respective R_f-values.

List of chemicals needed:		Safety codes:	
1,4-dimethoxybenzene	(400 mg)	R 36/37/38	S 26, 37/39
acetic acid	(2.0 mL)	R 35, 21	S 16, 45, 26, 36/37/39
tert-butyl alcohol	(1.0 mL)	R 20/22, 36/37/38, 41	S 16, 26, 36
conc. sulfuric acid	(0.5 mL)	R 49, 23, 34	S 45, 36/37/39, 23
methanol	(10 mL)	R 11, 23/25	S 7, 16, 24, 45
water	(20 mL)		
TLC plates (silica gel 60 F254)			

Safety: Concentrated H_2SO_4 must be handled with great care, read the safety rules for working with strong acids. In the event that you spill this acid on your hands or clothes, immediately rinse with running tap water.

Questions:

24-1 Which species is the electrophile in this alkylation?

24-2 Give the structure of the product when 1,3-dimethoxybenzene had been taken as the starting material.

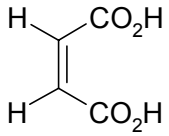
Note: Melting points can be determined in various ways, for instance, by using silicon oil in a round bottomed flask equipped with a magnetic stirrer and a thermometer or using a Kofler block

(metal block with holes to fit a thermometer and a capillary tube) or employing a Kofler hot bench (a metal alloy strip with gradient heating).

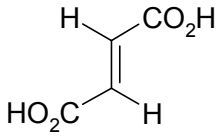
Problem 25 Experiment

Titration of maleic acid (*cis*-butenedioic acid)

Introduction.
Maleic acid is an unusual compound since the pK_a-values for the first and second dissociation are very different, viz. pK_a (1) = 1.8 and p*K*a (2) = 6.1. In contrast, fumaric acid, which is the corresponding *trans*-diacid, has pK_a-values that differ only slightly, viz. 3.0 and 4.4, respectively. In this experiment the first and second dissociation of maleic acid will be demonstrated by titration using different pH indicators, viz. phenolphthalein and methyl orange.



maleic acid



fumaric acid

Procedure 1

Maleic acid (290 mg) is dissolved in water (5 mL), 2 drops of phenolphthalein are added and the solution is titrated with 1.0 M NaOH until a clearly visible, permanent change of color has taken place.

Record the following data:
1. Volume (mL) of 1.0 M NaOH used.
2. Calculated amount of maleic acid in mmole.
3. Color before titration.
4. Color after completion of the titration.

Procedure 2

Maleic acid (290 mg) is dissolved in water (5 mL), 2 drops of methyl orange are added and the solution is titrated with 1.0 M NaOH until a clear, permanent change of color has taken place.

Record the following data:
1. Volume (mL) of 1.0 M NaOH used.
2. Calculated amount of maleic acid in mmole.
3. Color before titration.
4. Color after completion of the titration.

Questions

25-1 Determine on the basis of the titration results the minimal pK_a of:
a. phenolphthalein
b. methyl orange

25-2 The difference in the pK_a values of maleic acid can be attributed to:
- ❑ Intramolecular hydrogen bonding.
- ❑ Steric hindrance of the CO_2H group.
- ❑ Large dipole moment of the *cis*-acid compared to the *trans*-acid.
- ❑ Intermolecular hydrogen bonding.

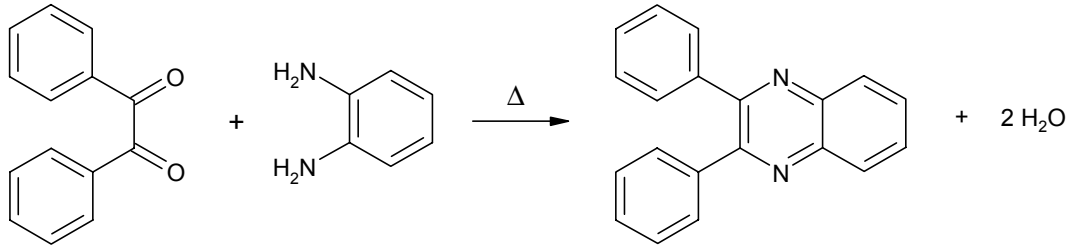
Mark the correct answer.

List of chemicals needed:		Safety codes:	
maleic acid	(600 mg)	R 22, 36/37/38	S 26, 28, 37
1.0 M NaOH	(20 mL)	R 34	S 26, 27, 28, 36/37/39
phenolphthalein	(a few drops)	R 36/37/38, 40, 43, 60	S 26, 36/37/39, 45, 53
methyl orange	(a few drops)	R 23/24/25	S 36/37/39, 45

Problem 26 Experiment

Preparation of 2,3-diphenylquinoxaline; an example of Schiff base formation leading to an aromatic compound

The reaction scheme for the preparation of 2,5-diphenylquinoxaline is as follows:



benzil

Procedure

Benzil (240 mg) and *ortho*-phenylenediamine (IUPAC name: 1,2-diaminobenzene)(216 mg) are mixed in a regular test tube and the mixture is heated in a hot water bath for 20 min. The mixture will first melt and then change into a light tan-colored solid. This solid is dissolved in hot methanol (ca 10 mL) and the solution is left undisturbed until crystallization starts. If crystallization fails to start, reheat the solution and add a small amount of water using a Pasteur pipette to the point of turbidity, crystallization will then begin, and will be completed on cooling to ambient temperature. The crystals should be filtered off as soon as they are formed, otherwise brown oxidation by-products may accumulate on standing too long. The product should appear as colorless needles. Weigh the product, calculate the yield and determine the melting point.

Record the following data

1. The weight of your product.
2. The yield of product as percentage of the theoretical yield (show your calculation).
3. The appearance and color of your product.
4. The m.p.

Note: The starting material 1,2-diaminobenzene is often colored, purification by sublimation prior to this experiment may be necessary.

List of chemicals needed:		Safety codes:	
benzil	(240 mg)	R 36/37/38	S 26, 36
1,2-diaminobenzene	(250 mg)	R 20/21/22, 36/37/38, 43, 45	S 26, 27, 28, 36/37/39, 45
methanol	(15 mL)	R 11, 23/25	S 7, 16, 24, 45
water			

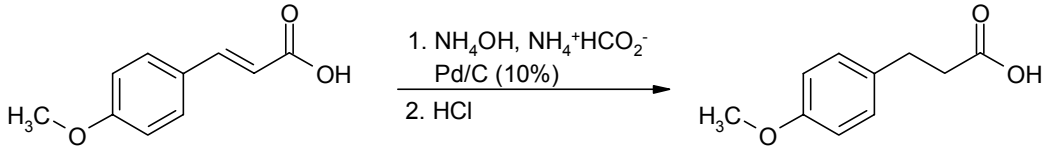
Question

26-1 Show the equation for the reaction of benzaldehyde ($C_6H_5CH{=}O$) with aniline (aminobenzene).

Problem 27 Experiment

Preparation of 3-(4-methoxyphenyl)propanoic acid; an example of a catalytic transfer-hydrogenation

The reaction scheme for the hydrogenation is as follows:



This hydrogenation in which ammonium formate serves as hydrogen source, is an alternative for the conventional catalytic hydrogenation using hydrogen gas. In both procedures Pd on charcoal is used as the catalyst.

Procedure

A magnetically stirred suspension of 4-methoxycinnamic acid (IUPAC name: *E*-3-(4-methoxyphenyl)prop-2-enoic acid) (450 mg) in water (7 mL) is treated slowly with concentrated aqueous ammonia (25%, 0.5 mL). After 5 min. palladium on charcoal catalyst (10%) and ammonium formate are added and the mixture is heated under reflux. The mixture gradually clears and the reaction is stopped after ca. 30 min. The completeness of the reaction is then checked by thin-layer chromatography (TLC) (silica gel 60 F254) plates using heptane / ethyl acetate / formic acid in the ratio 99 : 99 : 2 as the eluent. The starting material is used as reference. If starting material is still present, the reaction mixture is heated for another 10 min. Then the reaction mixture is cooled to room temperature and filtered through a Hirsch funnel covered with a small piece of filter paper. The filter is washed four times with water (0.5 mL). The clear solution is acidified with concentrated hydrochloric acid (10 M, ca 0.5 mL is needed to reach pH 2, use pH paper). A white precipitate is formed which is filtered off with a Hirsch filter, washed with water (10 mL) and partially dried. Whilst still slightly wet the product is taken up in heptane (10 mL) and the mixture is heated under reflux for 5 min. Then, the thus obtained colorless solution is decanted. The residue is extracted twice with heptane (5 mL). The combined decanted fractions are heated to completely dissolve all material. The product is then allowed to crystallize by cooling in a crushed ice bath. The crystals are collected by filtration, dried and weighed. The yield is calculated and the melting point is determined.

Note: This experiment can be carried out on half scale with the micro scale equipment described in this booklet.

Record the following data:
1. The weight of the product.
2. The yield of product in percentage of the theoretical yield (show your calculation).
3. The appearance of the crystals and their color.
4. The m.p.
5. Copy your TLC plate on the data sheet and calculate the respective R_f-values.

List of chemicals needed:		Safety codes:	
4-methoxycinnamic acid	(500 mg)	R 36/37/38	S 26, 37/39
ammonia (25%)	(1 mL)	R 36/37/38	S 26, 36
ammonium formate	(500 mg)	R 36/37/38	S 26, 36
Pd (10% on C)	(15 mg)	R 36/37/38	S 16, 24
heptane	(20 mL)	R 20/22, 36/37	S 7/9, 16,26, 33
ethyl acetate	(5 mL)	R 11, 23, 25	S 16, 24
formic acid	(0.5 mL)	R 25, 35, 42/43	S 23, 26, 36/37/39
conc. HCl	(1.0 mL)	R14, 34	S 23, 26, 27, 36/37/39
TLC plates (silica gel 60 F254)			
water			

Questions:
27-1 Calculate the quantity of starting material in mmoles.
27-2 Is ammonium formate used in excess? If so, calculate the molar quantity.
27-3 Propose a catalytic cycle for the transfer hydrogenation process. Hint: CO_2 is formed during the reaction.

Problem 28 Experiment

Complexometric titration; an example of metal ion determination using complexometry.

Introduction:
The concentration of Ni^{2+} ions can be determined by complexation with EDTA (ethylenediamine tetra-acetate). EDTA is a multidentate ligand which forms a 1 : 1 complex with Ni^{2+} ions. The indicator is murexide which also complexes with Ni^{2+} ions but this complex is less stable than with EDTA. The aim of this experiment is to determine the amount of crystal water in nickel sulfate.

Procedure

Nickel sulfate (ca. 300 mg) is weighed accurately and dissolved in water. Use a 100 mL volumetric flask. Make a buffer solution by dissolving ammonium chloride (2.7 g) and concentrated ammonia (17.5 mL) in water (50 mL). Fill a burette with EDTA standard solution (0.01 M). Transfer 10.00 mL of nickel sulfate solution with a pipette into an Erlenmeyer (200 mL) and dilute with water (ca. 90 mL). Add buffer (10 mL) while swirling the Erlenmeyer. Add a small amount of solid murexide indicator and ensure complete dissolution. Titrate with EDTA solution until change of color (yellow to purple). When the change of color is slow, add some concentrated ammonia at the end of the titration. This experiment should be carried out in duplicate.

Record the following data:

1. The amount of EDTA solution in mL. Record also the exact titer of this solution.
2. The weight of nickel sulfate.xH_2O.
3. Calculate the concentration of Ni^{2+} in solution.
4. Calculate the number of moles of crystal water per mole of nickel sulfate.

(Show the details of your calculations)

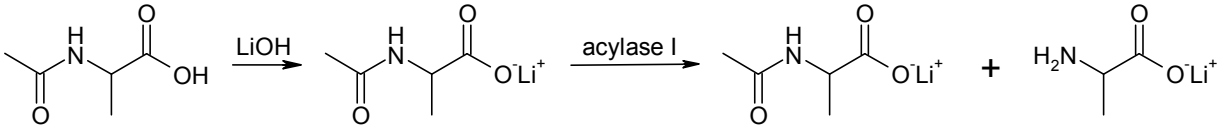
List of chemicals needed:		Safety codes:	
nickel sulfate	(300 mg)	R 20/21/22, 42/43, 45, 46	S 26, 27, 28, 36/37/39, 45
standard EDTA solution		R 22	S 36
murexide indicator		R -	S 22, 24/25
ammonium chloride	(3 g)	R 22, 36	S 22
concentrated ammonia	(20 mL)		
demi-water			

Problem 29 Experiment

Enzymatic hydrolysis of *N*-acetyl-alanine; an example of an environmentally benign process

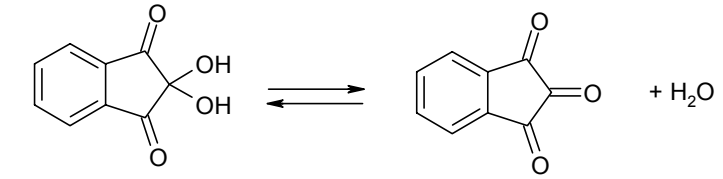
Introduction:

Biological reactions are catalyzed by enzymes. Many enzymes show a highly selective behavior and often are able to selectively catalyze reactions with one enantiomer of a racemate. In modern chemistry enzymes are used for many processes in vitro, especially for the synthesis of enantiomerically pure products. In this experiment the hydrolysis of *N*-acetyl-alanine with the enzyme *acylase I* is investigated. The reaction scheme is shown below:

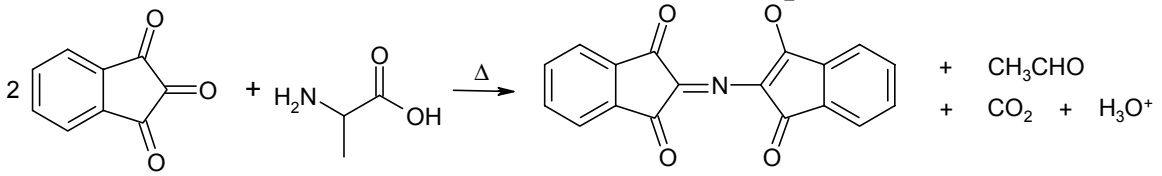


racemic *N*-acetyl-alanine — *N*-acetyl-alanine — alanine

The progress of the reaction can be monitored by following the formation of alanine using the reaction with ninhydrin, as shown below.



ninhydrin — indane-1,2,3-trione



alanine — purple anion λ_{max} = 592 nm

Procedure:

Racemic *N*-acetyl-alanine (262 mg, 2.0 mmol) is dissolved in water (10 mL). A solution of lithium hydroxide monohydrate (84 mg, 2.0 mmol) in water (4 mL) is then gradually added with gentle stirring. The pH is monitored with pH-paper until pH = 7 is reached. A solution of acylase I (10 mg) in water is added with vigorous stirring for 2 minutes. [This enzyme solution is prepared by adding the enzyme to 5 mL of water and filtration using a small glassfilter covered with diatomaceous earth]. Subsequently, water is added to reach the total volume of exactly 20.0 mL. The reaction mixture is kept at a temperature of 37 °C (use a water bath) for 60 minutes. Then an accurate volume of 0.25 mL (use a syringe or a fine measuring pipette) is transferred into a test tube and ninhydrin (Sigma N 1632), (1.25 mL) is added. This mixture is heated in boiling water during 20 minutes, whereby a deep purple color is developed. After cooling this mixture is accurately added to buffer solution consisting of 4 M lithium acetate aqueous buffer (pH = 5.2) and dimethyl sulfoxide in the ratio 1 : 3 in a volumetric flask of 250 mL. Adjust the volume to 250 mL. Then measure the absorption using a spectrophotometer at λ = 592 nm. Use as a reference ninhydrin in the same lithium acetate buffer in dimethyl sulfoxide. $\varepsilon_{\text{purple complex}}$ = 13350 L $mol^{-1}cm^{-1}$.

Record the following data:

1. The initial concentration of racemic *N*-acetyl-alanine.
2. The absorption at λ = 592 nm.
3. Calculate the amount in mmoles of alanine formed in the enzymatic reaction. Use the law of Lambert-Beer.
4. Calculate the percentage of conversion.

Questions:

29-1 Will the alanine formed be optically active, yes or no?

29-2 Will the *N*-acetyl-alanine that remains be optically inactive, optically enriched or optically pure when the conversion is smaller than 50%?

29-3 Idem when the conversion is 50% exactly?

29-4 Is a conversion larger than 50% possible, yes or no?

Special note:

If time permits you may decide to stop the reaction after 10, 25, 40 and 60 minutes and determine the concentration of alanine in each case. Then construct a graph of the concentration of alanine vs time, and estimate the optimal reaction time.

List of chemicals needed:		Safety codes:	
rac. *N*-acetyl-alanine	(265 mg)	R -	S -
acylase I	(10 mg)	R -	S -
lithium hydroxide	(85 mg)	R 23-34	S 45-26-27-36/37/39
ninhydrin solution (Sigma N1632)	(2 mL)	R 11-20/21/22-34	S 16-26-27-36/37/39
dimethyl sulfoxide	(ca. 70 mL)	R 36/37/38	S 26-36-23
lithium acetate buffer	pH 5.2	R 20/21/22-63	S 22-36