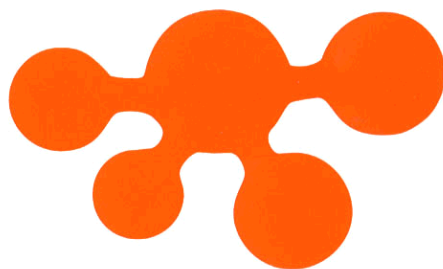


Name:

Student Code:



34th International Chemistry Olympiad

Groningen, Monday, 8 July 2002

Practical Examination

Chemistry and the Quality of Life go Hand in Hand

Enzymatic Hydrolysis of Methyl N-Acetyl-phenylalaninate

Synthesis of Benzylhydantoin

Determination of Iron in Iron Pills

Introductory Remarks

- At all times while you are in the laboratory you should wear safety spectacles or your own spectacles if they have been approved. Use only a pipette filler bulb for pipetting. Eating of any kind of food is strictly prohibited in the laboratory.
- Participants are expected to work safely, to behave socially and to keep equipment and work environment clean. Violation of these rules may result in penalty points. Do not hesitate to ask a laboratory assistant if you have any questions concerning safety issues.
- When you enter the laboratory, check the emergency exits and the place of the safety shower.
- Please carefully read the text of the entire experimental task and study the layout of the answer forms before you begin your experimental work. Check where instruments are located. You have 15 minutes to prepare yourself for the experimental tasks.
- Work may only begin when the start signal is given.
- You have **5** hours to complete all of the experimental tasks, and record your results on the answer sheets. There will be a pre-warning 15 minutes before the end of your time. You must stop your work immediately after the stop command is given. A delay in doing this by 5 minutes will lead to cancellation of the current task and will result in zero points for that task.
- **This practical examination comprises three experiments. In order to use the available time efficiently, it is necessary to make a work plan. Read the content of all three experiments carefully. Conducting experiments simultaneously may save a considerable amount of time.**
- Write your name and personal identification code (posted at your work station) in the appropriate box of the answer sheets.
- All results must be written in the answer boxes on the answer sheets. Data written elsewhere will not be marked. Do not write anything on the back of your answer sheets. If you need more paper for working or a replacement answer sheet, request it from the laboratory assistant.
- When you have finished the examination, you must put all papers into the envelope provided, then you must seal the envelope. Only papers in the sealed envelope will be marked.
- Do not leave the examination room until you have permission to do so. A receipt for your sealed envelope will be issued to you as you leave.
- Use only the tools and calculator provided.
- A copy of the Periodic Table of the Elements is provided.
- The number of significant figures in numerical answers must conform to the rules of evaluation of experimental error. The inability to perform calculations correctly will result in penalty points, even if your experimental technique is flawless.
- This examination has 5 pages of answer sheets.
- An official English-language version is available only on request.

Name:

Student Code:

Safety

The rules described in the Preparatory Problems “safety rules” should be followed strictly.

Disposal of waste chemicals, spills, and glassware

Organic filtrates and organic washings and any other waste should be placed in the waste beaker or bottle.

Use the appropriate waste containers for disposals of chemical and other waste materials.

Broken glass should be placed in the waste bucket.

Cleaning up

The lab bench should be wiped clean with a wet tissue.

Instructions for the Texas Instruments TL-83 plus calculator

The following instruction is sufficient for this Olympiad. This machine is a gift from Texas Instruments to mark this Olympiad. The calculator is able to perform many, many calculations, more than necessary for this examination. Other options can be found in the book, but do not use the book today.

On: Press on the button ‘ON’.

Off: First press the button ‘2nd’ and then press the button ‘ON’.

Adding, subtracting, multiplying and dividing is as usual:

e.g. adding: Number 1 + Number 2 ‘enter’

Brackets can easily be used (on the panel above 8 and 9 resp.).

The buttons for \ln , \log , x^{-1} and x^2 are on the panel.

For e^x first press the button ‘2nd’ and then the button ‘ln’ ; press the number and ‘enter’.

For 10^x first press the button ‘2nd’ and then the button ‘log’ ; press the number and ‘enter’.

For \sqrt{x} first press the button ‘2nd’ and then ‘x²’ ; press the number and ‘enter’.

For the number $e = 2.71828$ first press the button ‘2nd’ and then press the button ‘-:-’.

For the number $\pi = 3.14$ first press the button ‘2nd’ and then press the button ‘^’.

In general: The yellow functions can be activated by first pressing ‘2nd’ (yellow button) and then the desired function in yellow.

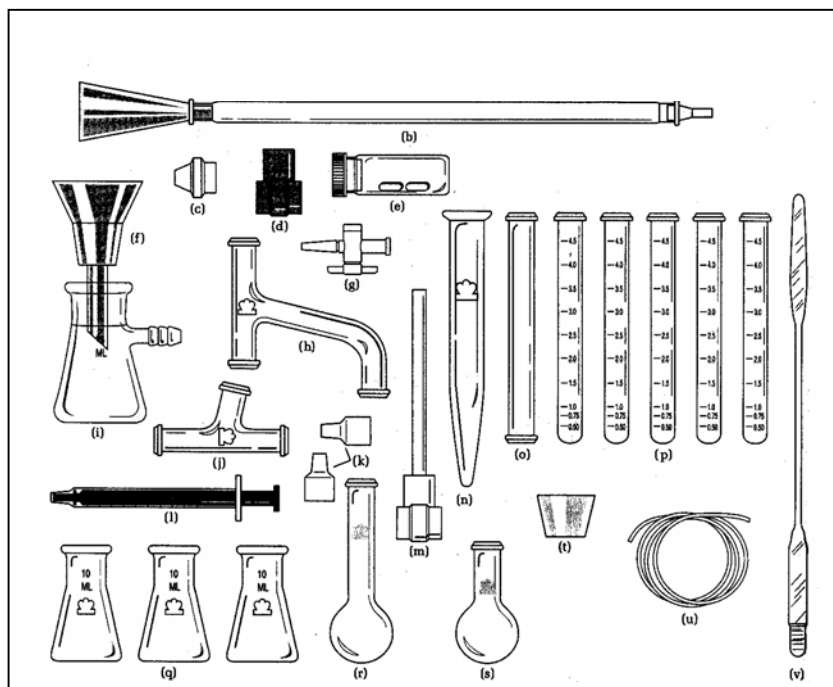
The screen can be cleared by pressing the ‘clear’ button.

Name:

Student Code:

Chemicals, glassware and equipment:**Microscale glassware kit**

- (a) Thermometer (on the bench)
- (b) Chromatography column
- (c) Thermometer adaptor
- (d) Connector
- (e) 2 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

**Glassware and equipment**

Sand bath (sand supplied separately)	1	
Erlenmeyer (50 mL)	1	
Burette (50 mL)	1	
Burette clamp	1	
Clamp holder with clamp	2	
Support stand	2	
Mortar and pestle	1	
Beaker 100 mL	1	
Measuring cylinder 10 mL	1	
Volumetric flask 250 mL	1	
Volumetric flask 100 mL	2	
Glass funnel	1	
Measuring pipette 10 mL	2	
Pipetting balloon	1	
Pasteur pipettes	10	
Pasteur pipette bulbs	3	
Weighing paper	20	(in location Zernicke near the balances)
Magnetic stirrer	1	
Magnetic stirring bar	1	(in location Zernicke in sample tube)
Pair of tweezers	1	
Spoon	1	
Screw cap bottle (large) for TLC	1	
Thin layer plates (5 _ 10 cm)	4	
Capillary tubes for TLC (in sample tube)	5	
Cuvets 1.000 cm	2	

Name:**Student Code:**

Stirring rod	1
Sample tubes	4
Stopwatch	1
Sealing bag	2

Chemicals

Methyl <i>N</i> -acetyl-phenylalaninate (NAcPheOMe)	500 mg (exact weight \pm 1 mg)
(<i>S</i>)-Phenylalanine (Phe)	500 mg (exact weight \pm 1 mg)
Sodium cyanate (NaOCN)	300 mg
α -Chymotrypsin solution (0.05% in water)	10 mL in a vial, available from the laboratory assistant
Iron pill in envelope	1 pill
Methanol (MeOH)	20 mL
Hydrochloric acid (HCl) 4 M	50 mL
Sodium hydroxide (NaOH) 0.1 M	70 mL (exact titer is given in your examination paper)
Sodium hydroxide (NaOH) 1 M	3 mL in small vial
Propyl red solution (0.02% in ethanol)	3 mL in small vial
Buffer solution pH=8	150 mL
Hydroxylamine.HCl solution (H ₂ NOH.HCl) 100 g L ⁻¹	10 mL
1,10-Phenanthroline solution 1 g L ⁻¹	20 mL
Di-isopropylether	50 mL
Acetone (high purity)	10 mL
TLC eluent (2% formic acid in ethyl acetate)	20 mL
pH-paper	4 pieces
Hi-flo filter aid	5 g
Wash bottle with acetone (for cleaning)	250 mL
Wash bottle with "demi" water	500 mL

Available for general use

Cleaning paper
Sponge
Brush
Waste container
Parafilm

Equipment for general use

Hotplate (only in location Zernicke)
Ultrasonic bath
Vacuumpump
Spectrophotometer
Balance
UV lamp

Name:

Student Code:

R and S phrases

Acetone

Formula	C_3H_6O
Molecular weight	58.08
Melting point	-95 °C
Boiling point	56 °C
Density	0.79 g/cm ³

- R11 Highly flammable
S9 Keep container in a well-ventilated place
S16 Keep away from sources of ignition
S23 Do not breathe vapour
S33 Take precautionary measures against static discharges



Di-isopropyl ether

Formula	$C_6H_{14}O$
Molecular weight	102.17
Melting point	-85 °C
Boiling point	68 °C
Density	0.72 g/cm ³

- R11 Highly flammable
R19 May form explosive peroxides.
R66 Repeated exposure may cause skin dryness or cracking
R67 Vapours may cause drowsiness and dizziness
S9 Keep container in a well-ventilated place
S16 Keep away from sources of ignition – No smoking
S29 Do not empty into drains
S33 Take precautionary measures against static discharges



Ethanol

Formula	C_2H_6O
Molecular weight	46.08
Melting point	-114 °C
Boiling point	78 °C
Density	0.78 g/cm ³

- R11 Highly Flammable
S7 Keep container tightly closed
S16 Keep away from sources of ignition



Ethyl acetate

Formula	$C_4H_8O_2$
Molecular weight	88.10
Melting point	-84 °C
Boiling point	76 °C
Density	0.90 g/cm ³

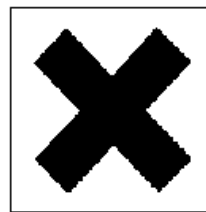
- R11 Highly flammable
R36 Irritating to the eyes



Name:

Student Code:

- R66 Repeated exposure may cause skin dryness or cracking
R67 Vapours may cause drowsiness and dizziness
S16 Keep away from sources of ignition – No smoking
S26 In case of contact with eyes, rinse immediately with plenty of water and seek medical advise
S33 Take precautionary measures against static discharges



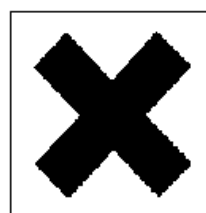
Hydrochloric acid

Formula HCl
Molecular weight 36.46
Density 0.909

- R11 Highly flammable
R37/37 Irritating to eyes, respiratory system and skin



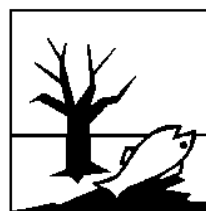
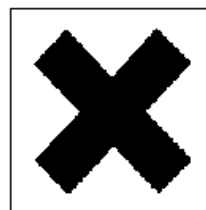
- 38
S16 Keep away from sources of ignition – No smoking
S26 In case of contact with eyes, rinse immediately with plenty of water and seek medical advise
S45 In case of accident or if you feel unwell, seek medical advise immediately (show the label where possible)
S7 Keep container tightly closed



Hydroxylamine hydrochloride

Formula $\text{H}_3\text{NO} \cdot \text{HCl}$
Molecular weight 69.49
Melting point $155\text{ }^\circ\text{C}$
Density 1.67 g/cm^3

- R22 Harmful if swallowed
R36/38 Irritating to eyes and skin
R43 May cause sensitisation by skin contact
R48/22 Harmful: danger of serious damage to health by prolonged exposure if swallowed
R50 Very toxic to aquatic organisms
S22 Do not inhale dust
S24 Avoid contact with skin
S37 Wear suitable gloves
S61 Avoid release to the environment.



Methanol

Formula CH_4O
Molecular weight 32.04
Melting point $-98\text{ }^\circ\text{C}$
Boiling point $65\text{ }^\circ\text{C}$
Density 0.79 g/cm^3

- R11 Highly flammable
R23-25 Toxic by inhalation, in contact with skin and if swallowed
R39/23 Toxic: danger of very serious irreversible effects through
24/25 inhalation, in contact with skin and if swallowed
S7 Keep container tightly closed



Name:

Student Code:

- S16 Keep away from sources of ignition –No smoking
S36/37 Wear suitable protective clothing and gloves
S45 In case of accident or if you feel unwell, seek medical advice immediately (show the label where possible)

1,10-Phenanthroline

Formula $C_{12}H_8N_2$
Molecular weight 180.20
Melting point 117-120 °C

- R25 Toxic when swallowed
R50/53 Very toxic to aquatic organisms, may cause long-term adverse effects in the aquatic environment
S45 In case of accident or if you feel unwell, seek medical advice immediately (show the label where possible)
S60 This material and its container must be disposed of as hazardous waste
S61 Avoid release to the environment



L-Phenylalanine

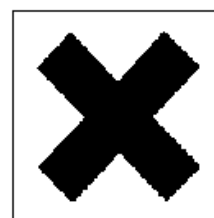
Formula $C_9H_{11}NO_2$
Molecular weight 165.19
Melting point 270-275 °C

- S24/25 Avoid contact with skin and eyes

Sodium Cyanate

Formula NaOCN
Molecular weight 65.00
Melting point 550 °C

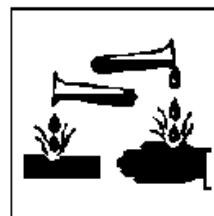
- R22 Harmful if swallowed
R52/53 Harmful to aquatic organisms, may cause long-term adverse effects in the aquatic environment
S24/25 Avoid contact with skin and eyes
S61 Avoid release to the environment



Sodium hydroxide

Formula NaOH
Molecular weight 40.00
Melting point 318 °C

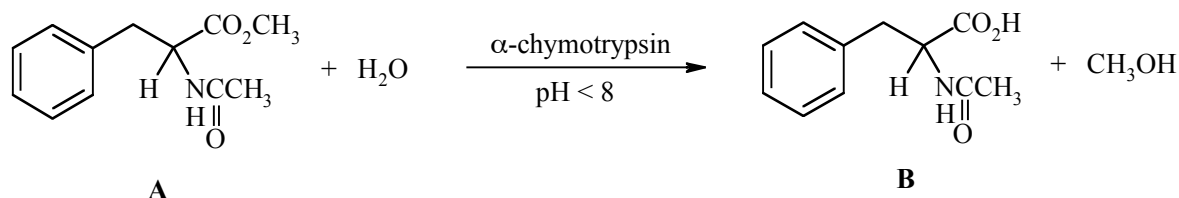
- R35 Causes severe burns
S26 In case of contact with eyes, rinse immediately with plenty of water and seek medical advice
S37/39 Wear suitable gloves and eye/face protection
S45 In case of accident or if you feel unwell, seek medical advice immediately (show the label where possible)



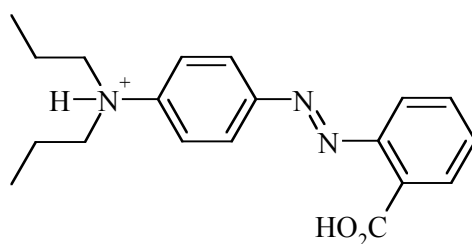
Enzymatic Hydrolysis of Methyl *N*-Acetyl-phenylalaninate

Introduction

α -Chymotrypsin, a protease enzyme recognizing derivatives of natural α -amino acids, catalyses the hydrolysis of esters. In this experiment the enzymatic hydrolysis of racemic methyl *N*-acetyl-phenylalaninate **A** is investigated (Scheme).



The rate of formation of *N*-acetyl-phenylalanine **B** can be monitored by titration with 0.100 M NaOH in the presence of propyl red as a pH indicator.



Propyl red (protonated form)
At pH < 5: pink; at pH > 6: yellow

Procedure

Note: the required amount of α -chymotrypsin will be supplied in a sample vial by the laboratory assistant on request.

Racemic methyl *N*-acetyl-phenylalaninate **A** [500 mg, the exact weight (± 1 mg) is indicated on the label of the vial marked as NacPheOMe] is transferred quantitatively into a 50 mL Erlenmeyer flask and dissolved in methanol (~ 2.5 mL). Subsequently, propyl red (0.02% solution in ethanol; 4 drops) is added. The kinetic experiment is started by adding α -chymotrypsin (10.0 mL of a 0.05% solution in distilled water) in one portion (*start the stopwatch*).

When the reaction mixture turns pink, it is immediately titrated with 0.100 M NaOH until the colour changes to yellow. When the pink colour reappears, add just enough titrant to restore the pale yellow colour, swirling the flask continually during the addition. You only need to record the reading on the burette every 5 minutes. (*Note: at the beginning colour changes occur very frequently.*)

Monitor the reaction for 75 minutes. A graph showing the amounts of NaOH consumed in mL versus time is constructed, in order to visualize the kinetic course of this enzymatic reaction.

Name:

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34th IChO Laboratory Task

Answer Sheet 1

Score 12 points

	1	2	3	4	5	6
Marks	10	30	30	10	10	10

Enzymatic Hydrolysis of Methyl *N*-Acetyl-phenylalaninate

1 Amount of the starting racemic methyl *N*-acetyl-phenylalaninate A
mg = mmol

2 Record the time in minutes and the total consumption of NaOH in mL (accuracy ± 0.05 mL), according to the scheme below. Final recording after 75 minutes.

Time (min)																	75
NaOH (mL)																	

3 Construct a graph of the total consumption of NaOH vs time on the supplied graph paper.
Put minutes on the x-axis: 5 min. per cm
Put mL NaOH on the y-axis: 1.0 mL per cm

4 Calculate the amount of 0.100 M NaOH consumed in this experiment in mmol
Answer:
Calculation:

Name:

Student Code:

Enzymatic Hydrolysis... (Cont'd)

Answer Sheet 2

5 Calculate the degree of hydrolysis of methyl *N*-acetyl-(*R,S*)-phenylalaninate **A** in mol%

Answer:

Calculation:

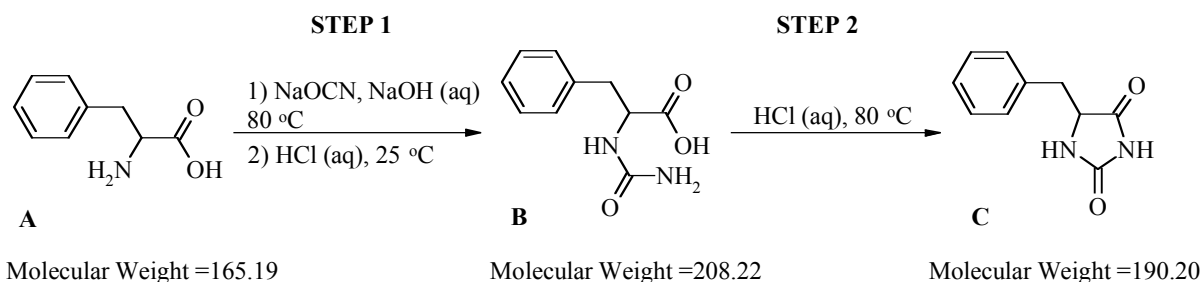
6 Which of the following statements is in accordance with your experimental results? Mark the appropriate box.

- The enzyme catalyses the hydrolysis to give methyl *N*-acetyl-(*S*)-phenylalaninate and *N*-acetyl-(*R*)-phenylalanine.
- The enzyme catalyses the hydrolysis to give *N*-acetyl-(*R,S*)-phenylalanine.
- The enzyme catalyses the hydrolysis to give methyl *N*-acetyl-(*R*)-phenylalaninate and *N*-acetyl-(*S*)-phenylalanine
- The enzyme loses its catalytic activity during the course of the reaction.

Synthesis of Benzylhydantoin

Introduction

α -Amino acids are the building blocks for peptides and proteins. They are also frequently used as starting material for the synthesis of pharmaceuticals. In this experiment natural *S*-phenylalanine **A** is converted in two steps into benzylhydantoin **C**, which is a useful intermediate for the preparation of various physiologically active derivatives.



Procedure

STEP 1

Retain a tiny amount of starting material **A** for the TLC analysis (see below). A long-necked round-bottomed flask is charged with (*S*)-phenylalanine **A** (500 mg, 3 mmol, the exact amount is indicated on the label of the vial), sodium cyanate (300 mg, 4.6 mmol), water (3 mL) and a stirring bar. Two drops of aqueous sodium hydroxide (1 M) are added to the stirred suspension. The flask is equipped with a condenser (distillation column) and the reaction mixture is heated to 80 °C on a sand bath while stirring magnetically.

Important

In order to reach the appropriate temperature in time and not lose too much time, start the electric heating of the sand bath immediately at the beginning of this experiment. Check the temperature of the sand bath regularly and carefully with a thermometer.

After heating the reaction mixture at 80 °C for at least 30 minutes, the resulting clear solution is cooled to room temperature and poured into a small Erlenmeyer flask. Rinse the round-bottomed flask with a little water. The solution is acidified by dropwise addition of hydrochloric acid (4 M) to pH < 3 with magnetic stirring. Some water is added to the resulting white suspension in order to facilitate stirring.

The white precipitate is then filtered off by suction, washed with ample water (on the filter) and then washed twice with a small amount of di-isopropyl ether to remove most of the adhering water. The urea derivative **B** is left on the filter under suction for at least 3 minutes to remove as much solvent as possible.

A small amount of the obtained urea derivative **B** is retained for TLC-analysis later.

STEP 2

The urea derivative **B** is now transferred into a long-necked round-bottomed flask and hydrochloric acid (4 M, 3 mL) is added. A stirring bar is introduced and the suspension is stirred thoroughly whilst heating at 80 °C on a sand bath. A clear solution is obtained. After a reaction time of 30 minutes, the reaction mixture, which may already contain some precipitate, is cooled to room temperature. The obtained suspension is filtered by suction, washed thoroughly with water and finally washed twice with a small amount of di-isopropyl ether. The product is left on the filter under suction for at least 3 minutes. It is then collected on a filter paper and dried in the air for at least 30 minutes.

Name:

Student Code:

The final product **C**, its precursor **B** and starting material **A** (see above) are subjected to TLC-analysis. For this purpose small amounts of either compound are dissolved in a tiny amount of pure acetone. Small samples of these solutions are applied to a TLC plate, using the supplied capillary tubes. The analysis is carried out with two TLC plates in one run. The TLC-plates are developed with a solution of 2% formic acid in ethyl acetate as the eluent. After the elution the TLC-plates are analysed using a UV-lamp. The starting line, solvent front and the UV-active spots are clearly marked with a pencil. Copy the diagram in the box on the answer sheet. The R_f values are determined. Finally, the TLC-plate with the best analysis is wrapped in parafilm and placed in a plastic bag with a sealing strip.

The final product **C** is transferred into a sample vial of which the empty weight has been pre-determined (weight is indicated on the label). Weigh the vial with product and calculate the yield of the product **C**.

The examination committee will check the quality of the benzylhydantoin that you have prepared by determining its melting point using an automatic melting point apparatus.

Name:

Student Code:

34th IChO Laboratory Task

Answer Sheet 3

Score 18 points

	1	2	3	4	5	6	7	8
Marks	10	20	10	10	20	10	10	10

Synthesis of Benzylhydantoin

Weight of your starting material **A** (see label on the vial):

mg

Weight of the empty sample vial:
(see label on the vial: YOUR PRODUCT)

mg

1 Weight of the sample vial containing your product **C**:

mg

2 Amount of benzylhydantoin **C** obtained:

mg

Calculate the yield of benzylhydantoin **C**:

Answer: %

Calculation:

3 R_f value of urea derivative **B**

Answer:

Calculation:

4 R_f value of benzylhydantoin **C**

Answer:

Calculation:

Name:

Student Code:

Synthesis of Benzylhydantoin (Cont'd)

Answer Sheet 4

5 Copy the TLC diagram in the box below

A

B

C

|
base line

also indicate the front
of the solvent

6 Conclusions from the TLC analysis:

Compound B:

- is pure
- contains some A
- contains several contaminants

Compound C:

- is pure
- contains some B
- contains some A and B
- contains several contaminants

7 Appearance of benzylhydantoin C, mark what is appropriate for your product.

- White colour
- Yellowish colour
- Sticky
- Crystalline
- Powder

8 Melting point of benzylhydantoin C will be determined later by the examination committee
- °C

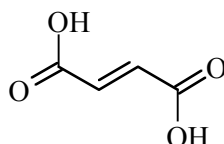
Place your packed TLC plate (see procedure) in an envelope with your name and student number.

Determination of Iron in Iron Pills

Introduction

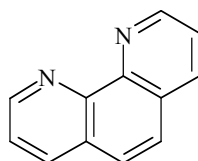
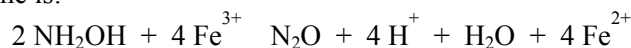
Iron is an essential component of hemoglobin, transporting oxygen in the blood to all parts of the body. It also plays a vital role in many metabolic reactions. Iron deficiency can cause anaemia resulting from low levels of hemoglobin in the blood. Iron deficiency is the most widespread mineral nutritional deficiency worldwide. One way to reduce iron shortage is by treatment with iron pills.

The active ingredient in the iron pill to be examined, is iron(II) present as iron(II) fumarate. Besides this organic iron(II) compound the pill contains other compounds such as binding agents. The structure of fumaric acid is:



Fumaric acid

Iron(II) and 1,10-phenanthroline form an orange/red coloured complex $[(C_{12}H_8N_2)_3Fe]^{2+}$. The absorbance of this complex, determined at 510 nm in a buffer solution (pH=8) is a measure for the iron content of the iron pill. Since 1,10-phenanthroline only binds to iron(II) and iron(II) is readily oxidized to iron(III), hydroxylammonium chloride is added to reduce all iron(III) to iron(II). A simplified reaction scheme is:



1,10-Phenanthroline

Procedure

The weight of the iron pill is determined with an accuracy of 1 mg using a balance. The pill is carefully pulverized in a mortar and transferred quantitatively into a 100 mL beaker with the aid of a small amount of distilled water. Hydrochloric acid (5 mL, 4 M) is added. The content of the beaker is heated up to approximately 60 °C on a hotplate. The solution turns a yellow colour.

The beaker is then placed in an ultrasonic bath for at least 5 minutes. The beaker is kept in place by styrofoam. The suspension is filtered by suction using a Hirsch funnel containing a small layer of moistened hi-flow filter aid pressed onto the filter. The hi-flow filter aid is washed with ample distilled water. The filtrate is carefully transferred into a volumetric flask (250 mL) and the final volume adjusted by adding distilled water and with regular mixing. An amount of 10 mL is pipetted from this solution and transferred into a volumetric flask of 100 mL. Again the volume is adjusted with distilled water while mixing the content of the flask.

From this solution, 10 mL is pipetted and transferred into a volumetric flask of 100 mL. Subsequently, 1,10-phenanthroline solution (10 mL) and hydroxylammonium chloride solution (1 mL) are added. Then the volume is adjusted with buffer solution (pH 8).

Name:

Student Code:

The absorbance of this solution is measured with a spectrophotometer at 510 nm against water as a blank in a 1.000 cm cuvet.

Calculate the amount of iron in the iron pill on basis of the known molar absorptivity (extinction coefficient, ϵ) of the iron(II)phenanthroline complex at 510 nm. The molar absorptivity of the iron(II)phenanthroline complex at 510 nm is $11100 \text{ M}^{-1}\text{cm}^{-1}$.

Important

In order to eliminate deviations in absorbance typically connected to the spectrophotometer used, a correction factor is denoted on the spectrophotometer you will be using for your experiment. The absorbance observed must be multiplied by this factor in order to obtain the correct absorbance of the solution of the iron complex.

Name:

Student Code:

34th IChO Laboratory Task

Answer Sheet 5

Score 10 points

	1	2	3	4	5
Marks	15	40	20	10	15

Determination of Iron in Iron Pills

1 Weight of the iron pill mg

Number of the spectrophotometer

Correction factor

2 Reading of the spectrophotometer: ; corrected absorbance: AU

3 Concentration of iron(II)phenanthroline complex in the cuvet: mmol L⁻¹
Calculation:

4 Total amount of iron(II) in the pill: mg
Calculation:

5 Calculate the iron content of the pill in weight%
Answer:

Calculation:

Name:

Student Code:



Periodic Table of Elements

Atomic weights based on $^{12}\text{C} = 12$
(Numbers) = most stable isotope

s block		d block Transition Metals										f block										p block																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																	
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