Learn Chemistry

First Year Undergraduate Chemistry Laboratory Course Manual 2011-2012

Core Chemistry 1A and 1B: Discovery Block 4

Developed by Dr Jacquie Robson, RSC School Teacher Fellow 2010-2011 at Durham University

This resource was produced as part of the National HE STEM Programme

National HE STEM Programme

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Core Chemistry 1A and 1B

First Year Chemistry Laboratory Course Manual 2011-2012

DISCOVERY BLOCK 4

Name ……

Core Chemistry 1A session:
Day/Time: …… Group name: ……..

Core Chemistry 1B session:
Day/Time: …… Group name: ……

Contents

Safety in the first year laboratory (CG 021)

The Health and Safety at Work Act was introduced in 1974. Since then many regulations have been made under the act, for example, The Control of Substances Hazardous to Health (COSHH). The University has a statutory obligation to comply with these requirements and you, as a student, have a duty to abide by the regulations. The following notes are to guide you in good laboratory practice and to familiarise yourself with the safety aspects of your laboratory work.

Emergency Telephone Numbers

Internal telephones:	FIRE, POLICE, AMBULANCE	9999
	UNIVERSITY EMERGENCY NUMBER	43333

1. Staff with special responsibilities for safety:

Chairman of the Board of Studies:	Professor J S O Evans
Chemistry Safety Officer:	Dr J A G Williams
Undergraduate teaching laboratories:	Dr E Wrede (Physical Chemistry)
	Dr J A G Williams (Inorganic Chemistry)
	Dr E Khosravi (Organic Chemistry)

2. No work is to be carried out unless a member of staff is present.

3. All persons in laboratories (whether or not they are actually doing practical work) must wear safety spectacles and laboratory coats. Academic staff supervising undergraduates enforce this rule. In all laboratories, hair should be secured so that it does not hang below the neck. It is important to wear suitable clothing, and your footwear must incorporate flat heels, slip-resistant soles and uppers fully enclosing the foot.

4. Foods, drinks, cigarettes, cosmetics and mobile phones must not be taken into or used in areas where chemical substances are used or kept.

5. Bags and coats should be placed in the lockers provided outside the laboratory and not left in corridors or on benches.

6. All accidents and dangerous occurrences must be reported immediately to a member of staff or a demonstrator. The first aid box is located in the foyer area and a list of qualified first aiders is on the front. The accident book is kept in room CG 058 and the member of staff in charge of the laboratory must fill out a report for all incidents. An emergency shower is located in the foyer area and there are four eyewash stations beside the sinks. There is a chemical spillage treatment kit in CG195.

7. The fire action signs in the laboratory indicate the nearest fire alarm and the emergency exit. There are two carbon dioxide fire extinguishers on either side of the central pedestal and another in the instrument room. There is also a foam spray fire extinguisher on either side of the central pedestal and one at each fire exit. A general fire practice is held twice yearly to check the smooth operation of the procedure so you should ensure that you know where to go in an emergency.

8. Pipetting by mouth is not allowed. Use a bulb or automatic pipette.

9. Do not inhale vapours or make skin contact with any substances. Use gloves where necessary always remembering that they are semi-permeable.

10. Experiments must be conducted on clean working surfaces; any spillage should be cleaned immediately. A high standard of tidiness should be maintained at all times. Contaminated surfaces and equipment must be cleaned as soon as it is practicable after use. The equipment should then be put away. Do not clutter bench-space with unused equipment and bottles of chemicals.

11. Waste should be disposed of in the appropriate containers: solvents should be placed in either the C, H, N, O-containing waste solvent bottles (Category C Waste), or halogen, sulphur-containing waste solvent bottles (Category D Waste). Heavy metal waste should be placed in the appropriate bottle. Broken glassware should be washed and placed in the designated glass bin. Solid waste should be dried, placed in a polythene bag and placed in a solid waste bin. A sharps bin is located in CG195. Consult a demonstrator if you are unsure about the correct disposal procedure.

12. The COSHH assessment of any chemical you use or make will be given in the laboratory script. There are further safety warnings at the appropriate parts of the text. Staff and student demonstrators reinforce these. If you are in any doubt, consult a demonstrator.

13. No unauthorised experiments are to be carried out.

14. It is important to ensure that hands are washed and all protective clothing removed **before** leaving the laboratory.

Introduction

Chemistry is an experimental science and, as well as attending lectures, both the University and the Royal Society of Chemistry, who accredit your degree, require you to complete a designated number of hours of laboratory work. During the first year, 18 weeks of practical work must be completed. The first year practical course is split into four sections:

1. Induction (Week 1)
2. Skills (Weeks 2-7)
3. Discovery (Weeks 8-16)
4. Projects (Weeks 18-19).

During Blocks 3, 4 and 5, you will complete the Discovery section. This contains activities designed to extend and build upon the key skills you have developed and practised in the Skills section. Some experiments will lead on from some in the Skills section, some will be linked to lecture courses you have studies and others will introduce new chemistry and ideas. Ideas developed in previous sections is now assumed knowledge, so you may need to refresh your memory by reading back through older laboratory manuals and your lab notebook as part of your pre-lab preparations.

1.1 The pre-lab exercises

As in the previous section, before every laboratory session one or more pre-lab exercises must be completed. These may involve reading, watching video clips, answering questions, completing assignments or using interactive software to rehearse techniques. Many of the files and resources for these exercises will be accessed via DUO, the university Virtual Learning Environment, which you should now be familiar with using regularly.

Pre-lab exercises will often contain summative aspects (i.e. the marks will count towards the overall marks for the Laboratory Course), and they must be completed in the week before you attempt the laboratory activity. All pre-lab work must be finished an hour before the relevant laboratory session so that completion can be checked. For example, a student attending the Thursday laboratory session, which begins at 9.00am, must have completed the pre-lab exercises by 8.00am that same day. Anyone arriving at a laboratory session without having completed the pre-lab exercises will be sent away to complete them before being allowed to begin work in the laboratory. Failure to complete the pre-lab exercises on time will incur a marks penalty. Your time in the laboratory will become very pressured if you are sent away to complete the pre-lab exercises. Good time management is the key to success in most areas of university life, but particularly in your laboratory work!

If there are any problems with access to DUO or LabSkills using personal computers, there are open-access machines available for use in the library and at other points around the science site. There may also be provision in college. Ask for help if problems arise when accessing the pre-lab exercises. Failure to access the exercises will not be accepted as a reason for incomplete pre-lab

work unless the laboratory course leader (Dr J. M. Robson) is informed in advance of the deadline so alternative arrangements can be made.

1.2 LabSkills

Many pre-lab exercises will again involve you using LabSkills. This is an electronic, interactive laboratory textbook for you to use to gain confidence in assembling and using apparatus before you begin work in the laboratory. Interactive exercises are designed to allow you to practice key techniques and learn more about apparatus and safety as you progress through the course. During the Discovery section of your laboratory course, the pre-lab exercises will be less prescriptive in their use of LabSkills but you should continue to use it as part of your pre-lab preparations to ensure you have refreshed your memory of the key techniques before your laboratory session. LabSkills also contains useful glossaries and worked examples of calculations that you will find useful. It will be accessible in the laboratory for additional assistance if you need it.

1.3 The laboratory sessions

One laboratory session per week will be assigned to Core Chemistry 1A, and a second session per week for Core Chemistry 1B. Experiments that will count towards Core Chemistry 1A will contain a suffix of 'A' in the title (e.g. Experiment 9A) and will be carried out by everybody. Experiment titles containing a suffix of 'B' (e.g. Experiment 9B) will count towards Core Chemistry 1B and will be carried out only by those studying Core Chemistry 1B.

In the second term (Michaelmas), University weeks begin on a Monday and end on a Thursday. Those students only studying Core Chemistry 1A will be assigned one laboratory session per week and will carry out all of the 'A' experiments. Those students also studying Core Chemistry 1B will be assigned two sessions per week. The first session of the week is assigned to be the Core Chemistry 1A session, and the second session of the week is the Core Chemistry 1B session. For example, if the two allocated laboratory sessions are on Monday morning and Thursday afternoon, the Core Chemistry 1A session will be Monday morning, when an experiment with the suffix 'A' will be performed, and the Core Chemistry 1B session will be Thursday afternoon, when the 'B experiment for that week will be completed.

Laboratory sessions will be allocated during one or two of the following times:

Monday	9.00am - 12.00pm
Monday	2.00pm - 5.00pm
Tuesday	2.00pm - 5.00pm
Wednesday	10.00am - 1.00pm
Thursday	9.00am - 12.00pm
Friday	9.00am - 12.00pm
Friday	2.00pm - 5.00pm

You may only attend the laboratory at your allocated time.

A risk assessment is either provided in this manual for the chemicals used in each experiment, or you will be asked to construct one before attending the laboratory. Each demonstrator will be able to advise on the hazards associated with each substance. Risk assessment advice must be followed throughout each laboratory session. All experimental work must be completed in that laboratory session and your lab notebook and work space signed off before you leave.

1.4 Set Allocation

Students in each laboratory session are allocated to one of three named sets of no more than 20 students. Sets are named after chemical elements and students are assigned to sets in no particular order. Lists showing members of each set are available on DUO and details should be written onto the front of the laboratory manual. During the second term (Epiphany), weeks begin on a Monday. This will necessitate some set changes within groups to ensure that students complete their 'A' experiment in their first session and their 'B' experiment in the second session. New set lists will become available before the start of the second term so students can check the experiment rota and identify the experiments they need to prepare for.

Each set will tackle a different activity each week, in a three week cycle, until everyone has completed each activity. The three experiments in the laboratory will then change and each set will again work through each experiment according to the rota.

Set names are as follows:

	Set 1	**Set 2**	**Set 3**
Monday am	potassium	lithium	molybdenum
Monday pm	niobium	phosphorus	osmium
Tuesday am	rhodium	strontium	tantalum
Wed am	uranium	vanadium	tungsten
Thurs am	actinium	bismuth	cobalt
Friday am	dysprosium	erbium	fluorine
Friday pm	gadolinium	hafnium	yttrium

Students will be allocated one set for their Core Chemistry 1A session and a second set for their Core Chemistry 1B session. Set lists are available on DUO and on the wall of the laboratory. Sets will perform experiments according to the following rota:

Week	**Set 1**	**Set 2**	**Set 3**
11	Experiment 11	Experiment 12	Experiment 13
12	Experiment 12	Experiment 13	Experiment 11
13	Experiment 13	Experiment 11	Experiment 12

For example, in week 12, everyone in Set 2 will carry out Experiment 13. Those studying only Core Chemistry 1A will only complete Experiment 13A. Those students also studying Core Chemistry 1B will complete Experiment 13B during their second session of the week. You should note your allocated set on the front of your laboratory manual.

1.5 Assessment

Pre-lab exercises will contain assessed components. Some will be marked by a demonstrator in the laboratory, others will be submitted for central marking. These exercises will differ between experiments. Completion of these exercises is compulsory and there will be a marks penalty for non-completion. The pre-laboratory exercise marks will make up 10% of the total marks for the practical course.

During each laboratory session, work and progress will be assessed. Completion of the lab notebook and performance in the practical tasks will be given marks. Occasionally there will be a small amount of post-laboratory work that will need to be completed to finish each experiment. Marks will be awarded during laboratory sessions throughout the year and will make up 10% of the total marks for the practical course.

In the Discovery section, a number of additional assessed components will be introduced in Experiments 10, 12 and 15 for Core Chemistry 1A and Experiments 10, 11 and 15 for Core Chemistry 1B.

1.6 Assessment summary (bold indicates activities to be completed during Skills)

	Core Chemistry 1A	**Core Chemistry 1B**
Pre-lab exercises (whole year)	**10 %**	**10 %**
Laboratory session marks (whole year)	**10 %**	**10 %**
SKILLS Experiment 5B: Determination of the enthalpy of vaporisation of ethanol	-	5%
DISCOVERY Experiment 10A	20 %	-
DISCOVERY Experiment 10B	-	15 %
DISCOVERY Experiment 11B	**-**	**20 %**
DISCOVERY Experiment 12A	**20 %**	**-**
DISCOVERY Experiment 15A	20 %	-
DISCOVERY Experiment 15B	-	20 %
PROJECT	20 %	20 % *
	100 %	**100 %**

*50 % of the project mark for students completing both Core Chemistry 1A and Core Chemistry 1B will be allocated towards the Core Chemistry 1A total marks, and 50 % will be allocated towards the Core Chemistry 1B total marks.

1.7 Supervising staff and postgraduate demonstrators

DISCOVERY Block 4 – week 11 to week 13

(Monday 16th January to Friday 3rd February 2012)

Senior Demonstrators (staff)	Junior Demonstrators (postgraduate students)
Dr Jacquie Robson*	Ffion Abraham
Dr Ezat Khosravi	Paul Brooks
Dr Ehmke Pohl	Rachel Carr
Dr Pippa Coffer	David Cole
	Lucy Clarke
	Matthew Didsbury
	Hayley Lumb
	Antonios Messinis

*laboratory course leader - email: j.m.robson@durham.ac.uk

DISCOVERY

BLOCK 4

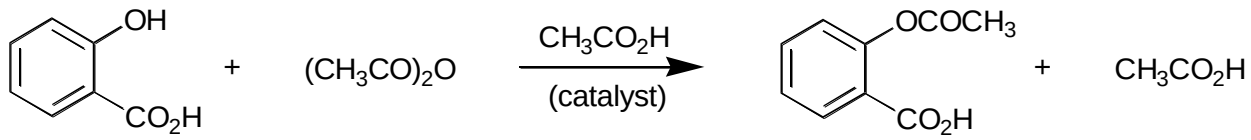
EXPERIMENT 11A

PREPARATION OF ASPIRIN

11A. Preparation of aspirin

Aspirin, also known as acetylsalicylic acid, is the most popular over-the-counter analgesic worldwide. In addition to its well-known painkilling effects, it is also an anti-inflammatory agent (reduces painful swelling), an anti-pyretic (reduces fever) and is thought to help prevent heart attacks.

It is readily prepared from 2-hydroxybenzoic acid, also known as salicylic acid, by the following reaction in which acetic anhydride in the presence of acetic acid is the acylating agent. An acylating agent is a species that introduces the R(C=O)- group to a substance during a reaction.



11A.1 Aims

- To prepare a sample of aspirin
- To test recrystallize the product
- To perform TLC (thin layer chromatography) to assess purity

11A.2 Pre-lab exercises

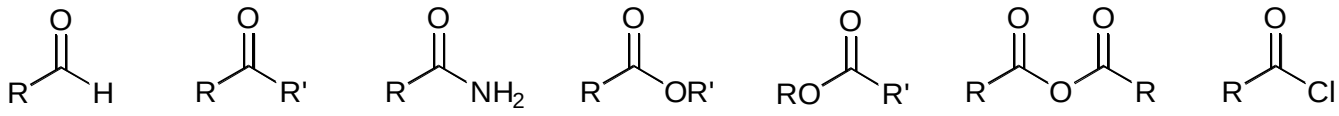
These exercises must be completed at least one hour before the timetabled start time of the laboratory session. Students not completing the pre-laboratory task will be turned away from the laboratory until the exercises are completed.

1. Read your laboratory instructions through carefully and highlight unfamiliar words or apparatus. Use text books, the internet, LabSkills or the Interactive Lab Primer to look up the meanings of these unfamiliar terms. If it is unfamiliar to you, be sure to read about TLC (thin layer chromatography). Read back over related experiments you have previously conducted, focusing particularly on how to set up and use reflux apparatus and how to recrystallize a solid.

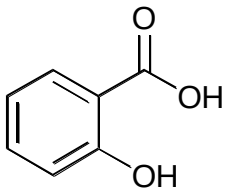
2. Prepare a risk assessment for the experiment in the same way as performed previously in Experiment 10A. Use previous risk assessment tables that have been provided in laboratory manuals as a template. The table should list all the chemicals encountered in the experiment (including solvents, starting materials and products), the R and S (Risk and Safety) numbers associated with that compound and the R and S phrases written out in full. Use the MSDS (Material Safety Data Sheet) documents to identify the appropriate R and S

numbers. Some have been provided on DUO. Others may need to be searched for on the internet. Cross reference these R and S numbers with the lists of phrases provided in DUO and copy them out in full into the table. Note that the R and S numbers are not normally given in your laboratory manual, but they should be included in your own risk assessments.

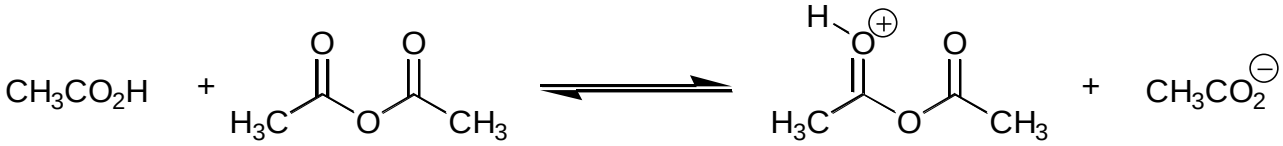
3. Study the following carbonyl-containing formulae. Draw those compounds which are *esters* in your lab notebook. Next, draw those which are *anhydrides*. Circle those compounds which are *isomers*.



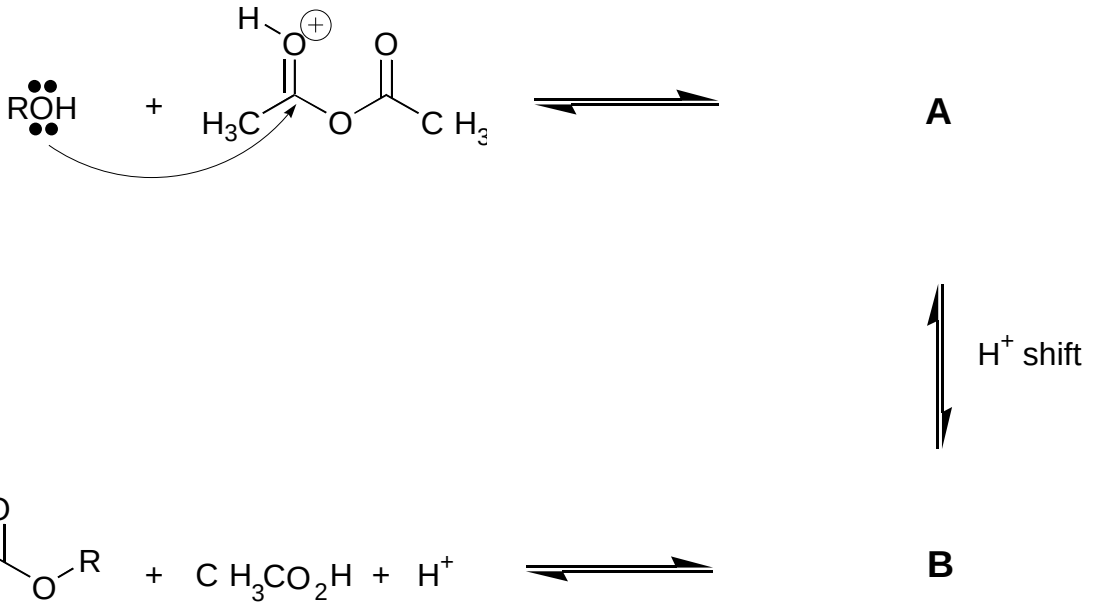
4. The structure of salicylic acid is shown below. Copy the structure into your lab notebook and show with labelled arrows which hydrogen atoms (H) could be replaced to make it contain TWO ester functional groups (using either an alkyl group, R, or an acyl group, RC=O).



5. Protons from acids, H^+, can form a bond with an electron pair on an atom. The function of the acetic acid in the acylation of salicylic acid with acetic anhydride is to make a carbonyl group even more electrophilic (more susceptible to nucleophilic attack):



Copy the following reactions (the mechanism of formation of aspirin) into the lab notebook, but draw in the structures of A and B. Draw in curly arrows to show formation of the products.



11A.3 Risk Assessment

You should have prepared your own risk assessment for this experiment as part of the pre-laboratory exercises, and it should be written neatly or printed and stuck in to your laboratory notebook when you arrive at the laboratory. This will be checked by a demonstrator before you begin work in the laboratory.

11A.4 Laboratory Activity

Work in pairs for this experiment, and work in a fume cupboard throughout. Work efficiently but work together so that observations can be recorded by both members of the team. Good team work and time management are essential to get this experiment completed in the time available. Choose a work area with a free locker and select a fume cupboard and note the numbers at the top of the lab notebook page for this experiment. **Show a demonstrator the lab notebook for marking and inform the demonstrator of the locker and fume cupboard number**. Use any breaks in the method (where time is needed to wait for recrystallization or for products to suck dry) to tidy up after previous steps or prepare future steps. Your ability to work well as a team will be assessed. You will need to work very quickly and efficiently to be sure to finish in this session.

Work in a fume cupboard throughout. Be sure to record all masses and observations in the lab notebook as the experiment proceeds.

1. Transfer 5.00 g of salicylic acid to a dry 100 ml B19 flask carefully. Using the pre-calibrated liquid dispensers in the fume cupboards, add 5 cm^3 of glacial acetic acid and 5 cm^3 of acetic anhydride. Add a magnetic stirrer bar.

2. Boil the mixture under reflux for 30 minutes using a stirrer hotplate and heating block. Cool the mixture with an external water bath. Once cooled, pour the reaction mixture into 100 cm^3 of ice-cold water in a beaker to precipitate the product. **Rinse the condenser and reaction flask immediately with water to minimise acetic anhydride and acid vapours**. If no precipitate forms, scratch the side of the beaker with a glass rod to induce crystallisation. **Show the product to a demonstrator.**

3. Filter the solid using a Buchner funnel and wash the solid thoroughly with cold water. Suck dry and press down the filter cake with a stopper. Retain a small sample (microspatula tip-full) of the dried crude solid for TLC analysis.

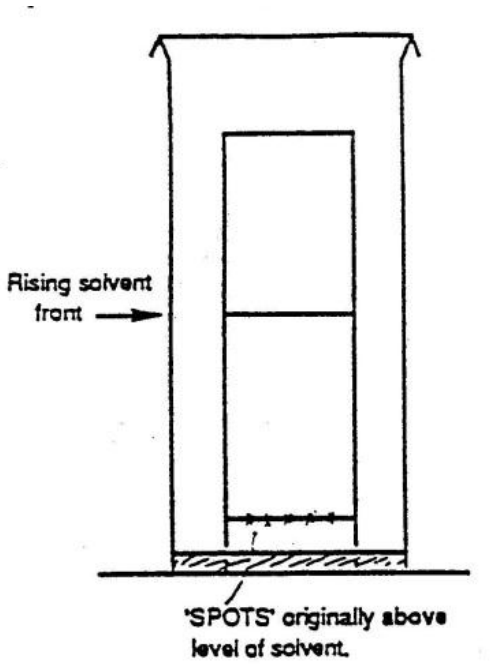
4. Recrystallise the remainder of the product in a 100 cm^3 round-bottomed flask fitted with a condenser using as solvent a 1:1 mixture of water and glacial acetic acid. The actual volume of solvent required will need to be determined during the experiment, but it is unlikely to exceed 15 cm^3. Do not perform a hot filtration unless there is insoluble material remaining in the hot solution. Transfer the solution from the 100 cm^3 round-bottomed flask to a clean beaker and allow the solution to cool down to room temperature for crystallisation to take place. Filter the crystals on a Buchner filter, wash sparingly with water and suck dry. Record the mass and calculate the percentage yield.

5. Perform thin-layer chromatography (TLC) on your product to assess its purity. To do this, prepare solutions of the recrystallised product, the crude product (i.e. before recrystallisation) and salicylic acid itself by dissolving a microspatula tip-full of each species in dichloromethane, using about 0.5 to 1 cm^3 of solvent. **Do not remove any dichloromethane from the fume cupboard.**

6. To prepare the TLC tank, insert a filter paper around the inside of a 250 cm^3 beaker (this may require tearing off a small segment of the paper or fold it to make it fit). In a fume cupboard, saturate the filter paper with the solvent to be used and add sufficient solvent to the beaker to give a depth of 2 to 3 mm. In this experiment the solvent to be used is a mixture of light petroleum ether and ethyl acetate (70:30 by volume). To apply the solution to the TLC plate, use a fine capillary (not a melting point tube) with a flat end and place a small spot of the solution ($\leq$2 mm diameter) in the middle of a line pencilled horizontally 8 mm from the bottom of the plate. Allow the solvent to dry, then place another spot on top of the first one. Follow the same procedure for each solution, maintaining a distance of around 5mm between spots, using a fresh section of capillary for each spot.

7. Place the plate under the UV lamp and view through the viewer. The spots should appear dark on the pale green plate. If no spots are visible, continue adding spots over the top on the same plate, this time spotting each compound more times (e.g. three each), then visualise again with the UV lamp.

8. Carefully place the TLC plate in the beaker and prop against the glass, not against the moist filter paper. Cover the top of the beaker with another filter paper and make the seal as air-tight as possible. The set-up should look like:



9. Allow the solvent to rise until it is 1 cm from the top of the plate, remove the lid and then the plate and immediately mark the height that the solvent reached with a pencil. Allow the solvent to evaporate from the plate in a fumes cupboard and visualise the spots on the plate with the UV lamp. Mark each spot by drawing around it with a pencil. Draw a representation of your TLC plate into the lab notebook. Determine the retention factor (R_f) of each spot by measuring the distance travelled by the spot from the baseline and the distance from the baseline to the solvent front. The R_f value for each spot is calculated from

$$R_f = \frac{\text{distance travelled by spot}}{\text{distance travelled by solvent}}$$

Use the TLC results to draw a conclusion regarding the extent of conversion of salicyclic acid into aspirin and the purity of aspirin following recrystallization. Note down the conclusion in the lab notebook.

10. If there are more than 45 minutes remaining, attempt to determine the melting point of the aspirin.

11. Wash up and tidy away all equipment. **Have the lab notebook marked by a demonstrator and show them the tidy workspace and fume cupboard before leaving the laboratory.**

DISCOVERY
BLOCK 4
EXPERIMENT 11B

THE KINETICS OF AN ENZYME-CATALYSED REACTION

11B. The kinetics of an enzyme-catalysed reaction

Enzymes are biological catalysts. In this experiment, a chemical reaction catalysed by the enzyme α-chymotrypsin will be explored.

11B.1 Aims

- To determine the rate constants for the α-chymotrypsin-catalysed hydrolysis of an ester using a spectrophotometer.
- To analyse data using a non-linear regression analysis in Excel.
- To work as a team and with the rest of the set to generate numerous data sets.
- To produce a concise post-lab report.

11B.2 Pre-lab exercises

These exercises must be completed at least one hour before the timetabled start time of the laboratory session. Students not completing the pre-laboratory task will be turned away from the laboratory until the exercises are completed.

1. Read the instructions in the laboratory manual through carefully and highlight unfamiliar words or apparatus. Use text books, the internet, LabSkills or the Interactive Lab Primer to look up the meanings of these unfamiliar terms. Read through the information on colorimetry in LabSkills and use the simulations to understand the basics behind the use of spectrophotometry. Prepare the lab notebook for the experiment.

2. Study the document entitled "11B The kinetics of an enzyme-catalysed reaction – background information", available via the Core Chemistry 1B Laboratory Course folder on DUO, and complete the following exercises in the lab notebook:
    a) Explain why this is reaction described as an example of homogeneous catalysis.
    b) Draw out the structure of the trimethylacetate ion and the 4-nitrophenoxide ion.
    c) Explain why is it possible to analyse this reaction using a spectrophotometer.
    d) Write down the Beer-Lambert law and the meaning of all terms in the lab notebook.

3. Prepare a risk assessment for the experiment in the same way as performed previously in Experiment 10A. Use previous risk assessment tables that have been provided in laboratory manuals as a template. The table should list all the chemicals encountered in the experiment (including solvents, starting materials and products), the R and S (Risk and Safety) numbers associated with that compound and the R and S phrases written out in full. Use the MSDS (Material Safety Data Sheet) documents to identify the appropriate R and S numbers. Some have been provided on DUO. Others may need to be searched for on the internet. Cross reference these R and S numbers with the lists of phrases provided in DUO

and copy them out in full into the table. Note that the R and S numbers are not normally given in your laboratory manual, but they should be included in your own risk assessments.

11B.3 Risk assessment

You should have prepared your own risk assessment for this experiment as part of the pre-laboratory exercises, and it should be written neatly or printed and stuck in to your laboratory notebook before you arrive at the laboratory. This will be checked by a demonstrator before you may begin work in the laboratory.

11B.4 Laboratory Activity

Work in fours or threes. Assign a team leader. The team leader should work with their team to ensure that the experiment is carried out efficiently, that all the preliminary parts of the experiment (Parts 1 to 3) are carried out quickly and that at least one enzyme-catalysed reaction is completed and analysed before the end of the session. Each team member will need to record all the data from their team's experiment, and each team will need to share final results from Part 4 with the other teams in the laboratory before leaving. This will allow multiple data sets to be analysed in the post-lab exercise.

Each team should choose a computer with a spectrophotometer from benches 2 or 3 and a workspace and locker on either of benches 1 or 4. Ensure that there is plenty of space to work. Members of the team should be allocated tasks to ensure parts 1 to 3 are completed quickly, and that part 4 is completed at least once during the session.

Part 1: Preparation of solutions

All solutions should be stored on the bench in sealed containers and clearly labelled.[1]

1. Solution #1: Take about 100 cm^3 of 0.01 M TRIS buffer (tris(hydroxymethyl amino) methane in water) which has a pH = 8.5. This solution will be used as the reaction medium and also as a solvent blank.

2. Solution #2: Take 25 cm^3 of a 3.4×10^{-3} M solution of the substrate, 4-nitrophenyl trimethylacetate, in acetonitrile.

3. Solution #3: Collect accurately about 50 mg of the enzyme α-chymotrypsin in 1.0 cm^3 of the pH 4.6 acetic acid-sodium acetate buffer. This solution will be used directly in the kinetic run. Record the mass of the enzyme used.

[1] All these solutions are water-based Category A waste, so may be disposed of by pouring down a fume cupboard sink whilst running the cold water tap.

4. Solution #4: Take 5 cm^3 of a 2.8×10^{-5} M 4-nitrophenol solution in the TRIS buffer. This is the yellow coloured solution on the bench. This solution will be used to determine the molar absorption coefficient of the product, P_1. Note that since the pK_a of 4-nitrophenol is ~7.0, the predominant species in the pH 8.5 TRIS buffer is the 4-nitrophenoxide ion.

Part 2: Determination of the molar absorption coefficient of 4-nitrophenoxide, the reaction product

1. On the computer attached to the spectrophotometer, open up the data acquisition software programme named Experiment 11B (double-click on the Experiment 11B icon, and then double-click on the Experiment 11B icon that appears).
2. Set the spectrophotometer wavelength to 400 nm, the position at which the absorbance of the hydrolysis product, P_1, is measured.
3. Record the temperature (try to ensure that it is 298 K).
4. Add TRIS buffer (solution #1) to a 1 cm spectrophotometer cell, place it in the spectrophotometer cavity, and zero the instrument. Remove the sample cell, rinse it and fill it with the 4-nitrophenol solution (solution #4). Replace the cell in the spectrophotometer, and record the absorbance.[2]
5. Determine the molar absorption coefficient of the 4-nitrophenoxide ion using the Beer-Lambert law and record the value in the lab notebook.

Part 3: Determination of the Spontaneous Hydrolysis Rate of the Substrate

This section allows determination of the rate constant of the uncatalysed (i.e. spontaneous) hydrolysis of 4-nitrophenyl trimethylacetate.

1. Fill a clean sample cell with precisely 3 cm^3 of TRIS buffer (solution #1).
2. Input the total running time as 1800 s and the data acquisition interval as 20 s in the appropriate boxes in the computer programme.
3. Start the programme by pressing the white arrow on the tool bar.
4. Add 100 μL of the 4-nitrophenyl trimethylacetate/acetonitrile solution (solution #2) to the sample cell, stopper the cell, invert it a few times, and place it in the spectrophotometer cavity. Immediately start collecting the absorbance data by pressing the green acquire button on the computer programme.
5. At the end of the 30-minute data collection a box will automatically appear prompting you to save your data. Save your data to an appropriate folder (use team leader initials and the day and date in the folder name e.g. JMRThu16-1-12 so that the folder is easily identifiable) on the server. Use an appropriate file name with a .txt extension and press Save. Do not stop the programme before the data acquisition time of 30 minutes is complete as the data

[2] Do not swap the 2 spectrophotometer cells with any other groups, as the cell pairs have been specifically matched.

will be lost. Do not try to save the data using File/Save as this will save a version of the programme and not the experiment data, and again, the data will be lost.

Part 4a: Running the enzyme-catalysed reaction

The concentration of enzyme, $[E]_0$, is constant for all experiments, only the concentration of substrate is varied.

1. Empty the sample cell from Part 3 on completion of all readings, rinse well with water and then with TRIS buffer (solution #1).
2. Add 30 μL of the enzyme stock solution (solution #3) to the cell followed by precisely 3 cm^3 of TRIS buffer (solution #1).
3. Place it in the spectrophotometer cavity and zero the instrument at 400 nm.
4. Add a volume (between 20 μL and 80 μL) of the substrate stock solution (solution #2) and mix thoroughly. Team leaders need to liaise with other teams to decide the volumes of solution #2 that each team will run. Each team should begin with a different volume.
5. Place the cell in the spectrophotometer and begin the data acquisition programme as before, recording the absorbance readings at exactly the same time intervals as used above for the spontaneous hydrolysis, i.e. every 20s for a total time of 30 minutes. Save the data as before.

If there is a spectrophotometer and computer not in use by another team, set up an additional enzyme-catalysed reaction run using a different volume of substrate stock solution (solution #2) while waiting for the data collection.

Part 4b: Analysis of the data set

1. Open the Experiment 11B Data Analysis file. A bar may appear stating that Macros have been disabled, click on Options … and Enable this content.
2. Make sure the cursor is in cell A1.
3. Insert your data into the worksheet by clicking on Data/Get External Data/From Text and change to the directory where your data is stored. Select your required .txt file, and then click on the Import button, use the Delimited/Tab/General choices that follow. The data should now be loaded into columns A and B in an Excel worksheet with a curve fitting button in it.
4. Multiply the absorbance values of the spontaneous substrate hydrolysis blank file by an appropriate factor that reflects the different substrate concentrations used. For example, if 100 μL were used in the blank and 80 μL used for the kinetic run, multiply the absorbances of the blank file by 0.8 before subtracting them from the kinetic data file. (This assumes that the spontaneous hydrolysis constant, k_0, is independent of concentration.)

5. Convert absorbance to concentration using the molar absorption coefficient of the product (4-nitrophenoxide ion) determined in this experiment.
6. Each of the data files (including the spontaneous substrate hydrolysis blank) has the same time interval between data points, and the same total acquisition time. Subtract the spontaneous hydrolysis blank from each of the kinetic runs.
7. Copy the time and corrected concentration data into columns I and J, respectively, with the first data points in cell 2. Then save the Excel file by pressing the file 'save as' button, changing the directory to the same one used to save the raw data, and inputting an appropriate file name.
8. Perform a non-linear regression analysis on the acquired P_1 vs. t data sets according to the equation $P_1(t) = Xt + Y\{1 - \exp(-Bt)\}$, by using the procedure outlined in Appendix 1.
9. At the end of the curve fitting procedure, values for X, Y, and B for each different initial substrate concentration, $[S]_0$, will be obtained. Tabulate the values of X, Y, and B as a function of $[S]_0$

If there are more than 45 minutes remaining before the end of the session, repeat Part 4a and 4b for a different volume of substrate solution (solution #2). Note that a separate Excel file must be used for analysing each different data set; it is not possible to use the macro on different data sets in different Sheets in the same file.

Once as many data sets as time allows have been collected, **dispose of all chemicals appropriately, wash up all equipment and put it away and have your work areas signed off by a demonstrator**.

Part 5: Sharing results

Values of X, Y and B are required for a number of different concentrations of substrate solution. Collect the required data from other teams before leaving the laboratory.

Show a demonstrator your lab notebook for marking before leaving the laboratory.

11B.5 Post-lab exercise – production of a concise laboratory report

Once the practical work is completed, the results should be analysed and an individual concise report produced. Guidelines for the production of the report are given below. It is recommended that the report is written immediately after the laboratory session finishes. **The report must be completed and submitted via DUO within one week of the end of the laboratory session** (i.e. the time that the laboratory session finishes is your deadline one week later). You are reminded that DUO time-stamps all submissions. Work submitted after your deadline will be awarded zero marks. Reports should be produced independently and not as a team.

11B.6 Report guidance

Reports should be prepared using Microsoft Word and Excel, should be concise and should be submitted electronically via DUO within one week of the end of the laboratory session. A paper copy of your submission should be stuck into your lab notebook to follow the notes from the experiment.

It is expected that you will refer to the background information document provided on DUO (and studied during the pre-laboratory exercises) to assist with the production of the post-lab report for this experiment. Other resources can be used to assist (e.g. text books, internet resources) but these must be referenced in your work. Remember also to look back over previous relevant sections of your laboratory manuals and your lab notebook from earlier in the laboratory course for assistance.

Be sure to include:

1. Experiment title and reference, your name, laboratory team names, experiment date.
2. A concise summary of the experiment (what was done and why).
3. Tabulated data in an appropriate format, as recorded during the laboratory session. Include values of $[E]_0$ (the molarity of α-chymotrypsin in pH 4.6 buffer in mol dm^{-3}), the absorbance of 2.8×10^{-5} M 4-nitrophenoxide ion in TRIS buffer at 400 nm, the molar absorption coefficient of the 4-nitrophenoxide ion at 400 nm and the temperature of the solution in the spectrophotometer. Give each table a Table caption. Microsoft Word can insert these automatically.
4. A plot of $1/\sqrt{Y}$ as a function of $1/[S]_0$ (equation 15 in background document) to obtain values of $k_3K/(k_2\sqrt{[E]_0})$ and $(k_2+k_3)/(k_2\sqrt{[E]_0})$ from the slope and intercept. Determine $K/(k_2 + k_3)$ and $1/(k_2 + k_3)$ from the slope and intercept of the double-reciprocal plot of equation 16 in the background document. Calculate individual values of K, k_2 and k_3 from these values.
5. Any Excel-produced graphs should be titled, fully labelled, clear and well formatted. Give each graph a Figure caption (e.g. 'Figure 1: A graph to show…'). Microsoft Word can insert these automatically. If you need help in producing graphs using Excel, see the University CIS 'Introduction to Excel 2007: Simple Formulae and Charts' document (Chapters 6, 7 and 8). This can be found at http://www.dur.ac.uk/resources/its/local/er-guides/ER004.pdf. This link is available via DUO.
6. A brief conclusion on your final results, giving reference to tables and figures (e.g. Figure 2 shows that… which implies that that…). What did you find out? What does this mean? Have you made an assessment of any random or systematic errors in the experiment? Be sure to report values of $(k_2+k_3)/k_2$, $k_2 + k_3$, k_3K / k_2 and the individual values of K, k_2 and k_3 obtained from your graphs.

7. A brief narrative statement of the outcome of any linear regression error analysis (i.e. give the gradient and/or intercept and their errors) and the source of errors. Do not include a copy of the full regression analysis output from Excel.
8. Be sure to correctly state units, and quote numbers to an appropriate number of significant figures. "Appropriate" is determined by the equipment and the regression analysis.
9. References used to assist in the production of the report.

Feedback on your submission will be provided at a later date via DUO. Remember that this submission will count 20% towards your total practical mark for Core Chemistry 1B. Remember that failure to submit the work by the deadline will result in a mark of zero being awarded unless the appropriate procedures have been followed well in advance of the deadline (see p39 of the First Year Handbook).

Useful information:

Molecular weight of α-chymotrypsin = 24 800 g mol^{-1}

Suggested Further Reading

1. R.A. Alberty and R.J. Silbey, *Physical Chemistry*, 2nd ed., pp. 730-736, Wiley (New York), 1977.

2. P.W. Atkins, *Physical Chemistry*, 6th ed., pp. 783-784, Oxford University Press (Oxford), 1994.

This list is not intended to be exhaustive; other text books and resources can and should be used, if required.

Appendix 1: Non-linear curve fitting in Excel

Non-linear curve fitting is a common scientific procedure. The basis of the procedure is to minimise the difference between the data and some model to describe the data, over the entire data range. The following instructions assume that the first time data point is in cell I2, and the first corrected concentration data point is in cell J2.

1. In cell P2, type **X**, in cell P3, type **Y**, in cell P4, type **B**.

2. Name cell Q2 'X', cell Q3 'Y', cell Q4 'B'. (To name a cell, firstly make the cell active by clicking on it, then click on *Formula*, then *Define name*. Finally type the desired name in the space provided. The default name will usually be the text entered into the cell to the immediate left of the selected cell.)

3. Plot columns I and J on an XY scatter graph, with column I as the x-axis values.

4. Enter initial guesses for X, Y, and B into cells Q2, Q3 and Q4. ***Hints*** the final gradient provides a good initial guess for X and a good approximate Y value is the absorption value found from extrapolating the final slope back to the y-axis. Set B = 0.01 initially.

5. In cell K2, type =(X*I2)+(Y*(1-exp(-B*I2))), and copy to cells K3:K91. This column will now contain the model data set that will depend on the values of X, Y and B.

6. Plot columns I and K on the same XY scatter graph as your experimental data, with column I as the x-axis values. This will enable you to compare the experimental data with the model.

7. In cell P7, type rsq. Name cell Q7 'rsq'.

8. Vary the values of X, Y and B until the match between the model and the data is reasonable. The closer the initial guess the faster the curve fitting will work. If the initial guess is too far from the actual values the curve fitting will fail. Do not be afraid to experiment; curve fitting will not affect your raw data, and you can always start again by restoring your saved data!

9. Once you are reasonably happy, press the 'Curve Fit' button. You will first be asked to select the range of cells containing time data, the experimental absorbance and the theoretical absorbance. This can either be done typing the appropriate range into the box (e.g., I2:K91) or highlighting the full range with the mouse. The value in Q7 is a measure of progress. The best fit corresponds to rsq = 1, as long as rsq is increasing the fitting procedure is working. You can cancel at any time by pressing the 'terminate' button – this will save the most recent values of X, Y and B, so you can restart the fitting from these values if you wish. Save the Excel file when you obtain a good fit.

DISCOVERY

BLOCK 4

EXPERIMENT 12A

STEREOCHEMISTRY

12A. Stereochemistry

This experiment is a 'dry' practical. Details will be provided on the day you complete the experiment. Work will be completed on sheets provided in the laboratory, and collected in at the end of the session and marked. This is a summative exercise, but no post-lab work is required. **It is essential that you bring your model kit to this laboratory session or you will place yourself at a disadvantage with the exercises.**

12A.1 Aims

- To complete a written exercise using a model kit to develop understanding of basic stereochemical principles.
- To convey 3-D information in 2-D by use of accepted structural drawing conventions.

12A.2 Pre-lab exercises

These exercises must be completed at least one hour before the timetabled start time of the laboratory session. Students not completing the pre-laboratory task will be turned away from the laboratory until the exercises are completed.

1. Revise stereochemistry from Core Chemistry 1A organic chemistry lectures and your text books (e.g. the relevant sections in Chapter 24 of Housecroft 4th Edition, or others). Read up particularly on chirality, isomerism, the use of sawhorse projections, Newman projections, assigning R and S configurations in chiral molecules, the meaning of 'diastereomer' and 'topism' and the meaning of 'homotopic', 'enantiotopic' and 'diastereotopic'.

You are not required to prepare anything in the lab notebook for this experiment. You may, if you wish, prepare some notes on the relevant topics in your lab notebook before the session, but this is not compulsory. You may also bring relevant text books with you. Remember to bring your model kit.

12A.3 Risk Assessment

This experiment has minimum risk, but the activity will be carried out in the laboratory and will require the wearing of usual PPE.

12A.4 Laboratory Activity

Attend the laboratory as normal, bringing lab coats, safety specs, pencils, pens, rulers, erasers and model kits. Each worksheet should be completed individually, but group discussion and discussion with the demonstrator is permitted. Text books may be referred to, if desired or required.

The time available for this activity is 3 hours. Named worksheets must be completed and handed in by the end of the session.

DISCOVERY
BLOCK 4
EXPERIMENT 12B

PREPARATION OF AN IODIDE OF TIN

12B. Preparation of an iodide of tin

In this experiment a sample of a tin iodide will be prepared and its molecular formula determined. A known mass of iodine will be allowed to react with an excess of metallic tin. The amount of tin that has reacted will be determined by weighing the metal before and after the reaction. Since the quantitative aspect of the experiment is based on the assumption that all the iodine used reacts with the tin, any loss of iodine, through spillage or as vapour, must be avoided.

For this experiment it is essential that the reaction flask and inner surface of the condenser are free of moisture. Iodine sublimes readily and if you use a flask straight from the oven significant quantities of iodine vapour will be formed, and the quantitative aspect of the experiment is lost.

12B.1 Aims

- To prepare a tin iodide using anhydrous conditions.
- To deduce the formula of the tin iodide.

12B.2 Pre-lab exercises

These exercises must be completed at least one hour before the timetabled start time of the laboratory session. Students not completing the pre-laboratory task will be turned away from the laboratory until the exercises are completed.

1. Read your laboratory instructions through carefully and highlight unfamiliar words or apparatus. Use text books, the internet, LabSkills or the Interactive Lab Primer to look up the meanings of these unfamiliar terms. Read back over any related experiments you have previously conducted, focusing particularly on how to set up and use reflux apparatus and how to calculate empirical formulas.

2. Prepare a risk assessment for the experiment in the same way as performed previously in Experiment 10A. Use previous risk assessment tables that have been provided in laboratory manuals as a template. The table should list all the chemicals encountered in the experiment (including solvents, starting materials and products), the R and S (Risk and Safety) numbers associated with that compound and the R and S phrases written out in full. Use the MSDS (Material Safety Data Sheet) documents to identify the appropriate R and S numbers. Some have been provided on DUO. Others may need to be searched for on the internet. Cross reference these R and S numbers with the lists of phrases provided in DUO and copy them out in full into the table. Note that the R and S numbers are not normally given in your laboratory manual, but they should be included in your own risk assessments.

3. Using text books, the internet or other resources, read about and make some notes in the lab notebook about known compounds of tin and iodine, including their colour and properties, to aid identification of the compound to be prepared in the laboratory session.

12B.3 Risk Assessment

You should have prepared your own risk assessment for this experiment as part of the pre-laboratory exercises, and it should be written neatly or printed and stuck in to your laboratory notebook when you arrive at the laboratory. This will be checked by a demonstrator before you begin work in the laboratory.

12B.4 Laboratory activity

Work in pairs for this experiment, and work in a fume cupboard throughout. Work efficiently but work together so that observations can be recorded by both members of the team. Choose a work area with a free locker and select a fume cupboard and note the numbers at the top of the lab notebook page for this experiment. **Show a demonstrator the lab notebook for marking and inform the demonstrator of the locker and fume cupboard number**. Your ability to work well as a team will be assessed.

Work in a fume cupboard throughout. Be sure to record all masses and observations in the lab notebook as the experiment proceeds.

1. Using a weighing bottle weigh out about 5 g of iodine (using an appropriate balance), and transfer to a cold and dry 100 cm^3 round-bottom flask. Ensure that the exact mass of iodine is recorded.

2. In a fume cupboard measure out 50 cm^3 of chloroform and add it to the flask; shake gently to dissolve part of the iodine. Attach the condenser to the flask, and heat the contents of the flask until a gentle reflux is obtained. A temperature of approximately 85^oC should be sufficient.

3. Meanwhile, clean the tin foil by wiping both sides with a piece of filter or tissue paper dampened with acetone. Weigh out about 4 g of the tin foil, ensuring that the exact mass of tin is recorded. Cut the foil into strips about 5 mm wide and roll these around a thin pen or pencil into a narrow extended coil, to look like an extended spring. The diameter of these springs should be small enough to allow them to travel unhindered all the way through the condenser to be used for the experiment. Do not cut the tin into small pieces because at the end of the reaction the residual tin must be removed and reweighed.

4. Once most of the iodine has dissolved, remove from the heat and allow the contents of the flask to cool slightly so that reflux stops. Add the small coils of tin down through the

condenser. Alternatively, use a paper tissue to remove condensation from the outsides of the neck of the flask and the lower part of the condenser, then remove the condenser and quickly add the weighed tin foil to the flask, replacing the condenser quickly, to avoid loss of iodine vapour. Water condensation from the apparatus should not be allowed to enter the flask. This will have a profoundly detrimental effect on the reaction.

5. Reheat the flask until a steady reflux is obtained. Continue heating until all the iodine has reacted. At this stage the drops of solvent at reflux should have become colourless, with the colour of the solution being a clear orange.

6. At the end of the reaction carefully decant the hot reaction solution into a warm beaker so that the residual tin remains in the flask. Allow the solution to cool slowly to room temperature so that crystals develop and grow.

7. While you are waiting for your product to crystallise, wash the residual tin with 3-4 small portions (approximately 5 cm^3) of chloroform (with warming and in a fume cupboard) to remove product from the tin. Continue the washing procedure until the tin is essentially free of adhered product, then air dry the tin in a fumes cupboard. Note the appearance of the metal, then reweigh using an appropriate balance.

8. If crystallisation does not occur, place the beaker on a stirrer hotplate at a moderate temperature (in a fume cupboard), add a stirrer bar and slowly remove some of the solvent by evaporation. Remove from the heat as soon as crystals start to appear at the surface of the solution. Isolate the crystals by suction filtration. Do not let the product come into contact with any moisture and do not wash with water, alcohols or acetone as it will decompose.

9. Place all the chloroform wastes in the bottle labelled ‘D-waste’. Do not add other solvents (or solvent mixtures) to this bottle. Once you have washed, dried and weighed the residual tin it may be disposed of as normal solid wastes.

10. Determine the empirical formula of the tin iodide. Assuming that the molecular formula is identical to the empirical formula, write a balanced equation for the formation of the tin iodide. Calculate the % yield of your product. Place your product in an appropriately labelled, sealed sample bag. **Hand in your sample to the demonstrator and show them the lab notebook for marking.**

11. Ensure all equipment has been cleaned and tidied away, and that all work areas have been left clean and tidy. **Sign out with a demonstrator**.

DISCOVERY
BLOCK 4
EXPERIMENT 13A

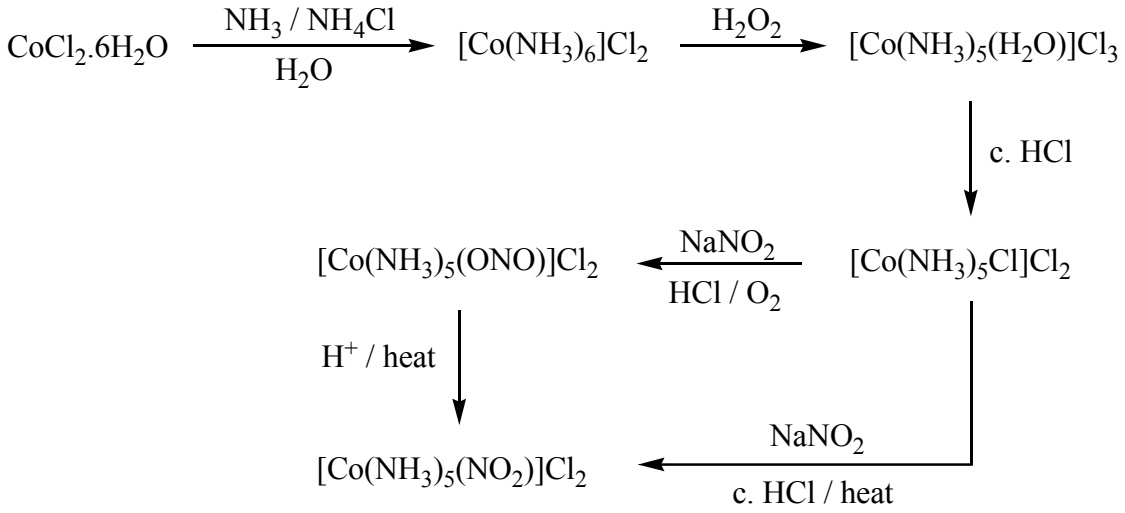
TRANSITION METAL COMPLEXES OF COBALT(II)

13A. Transition metal complexes of cobalt(II)

One of the features that make coordination compounds of, in particular, the d-block metals so important is their so-called 'complex' behaviour, something first rationalised by Werner. He studied many aspects of coordination chemistry, but his most notable contribution is to the understanding of the types of isomerism possible in such metal complexes, something that will be explored here.

In this experiment, linkage isomers will be prepared. Linkage isomerism is the type of structural (constitutional) isomerism which can occur when ambidentate ligands form complexes. Ambidentate ligands have two or more different sites that can be used for attachment to a metal ion, but usually only one is used at any given time. This experiment is concerned with the NO_2^- group, which bonds either through oxygen or nitrogen when it occupies one coordination position at a transition metal centre. When it bonds through nitrogen it is termed a nitro ligand and when it bonds through oxygen it is termed a nitrito ligand. Two such complexes, $[Co(NH_3)_5\underline{N}O_2]Cl_2$ and $[Co(NH_3)_5\underline{O}NO]Cl_2$, are prepared in this experiment.

The reaction sequence that will be used is as follows:



13A.1 Aims

- To prepare and compare complexes of cobalt, including nitro- and nitrito isomers.
- To develop understanding of some different ways ligands can bond to metal centres.

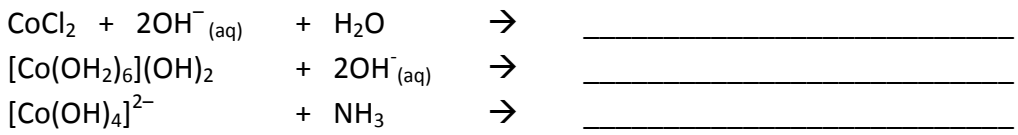
13A.2 Pre-lab exercises

These exercises must be completed before the experiment, and completed at least one hour before the timetabled start time of the laboratory session. Students not completing the pre-laboratory task will be turned away from the laboratory until the exercises are completed. Remember that questions posed in the laboratory manual can also be answered before the laboratory session, and calculations and equations prepared, to enable work in the laboratory to be more efficient.

1. Read the instructions in the laboratory manual through carefully and highlight unfamiliar words or apparatus. Use text books, the internet, LabSkills or the Interactive Lab Primer to look up the meanings of these unfamiliar terms.

2. Prepare a risk assessment for the experiment in the same way as performed previously in Experiment 10A. Use previous risk assessment tables that have been provided in laboratory manuals as a template. The table should list all the chemicals encountered in the experiment (including solvents, starting materials and products), the R and S (Risk and Safety) numbers associated with that compound and the R and S phrases written out in full. Use the MSDS (Material Safety Data Sheet) documents to identify the appropriate R and S numbers. Some have been provided on DUO. Others may need to be searched for on the internet. Cross reference these R and S numbers with the lists of phrases provided in DUO and copy them out in full into the table. Note that the R and S numbers are not normally given in your laboratory manual, but they should be included in your own risk assessments.

3. Considering Part 1 of the experiment, answer the following questions in the lab notebook:
   a. Write an equation to represent what happens when ammonia is dissolved in water.
   b. Why is this important in the first stage of the synthesis in Part 1?
   c. Copy and complete the following equations that describe the formation of $[Co(NH_3)_6]^{2+}$ (remembering to balance them):

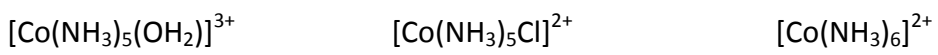


$CoCl_2 + 2OH^-_{(aq)} + H_2O \rightarrow$ _____

$[Co(OH_2)_6](OH)_2 + 2OH^-_{(aq)} \rightarrow$ _____

$[Co(OH)_4]^{2-} + NH_3 \rightarrow$ _____

   d. What are the oxidation states of cobalt in the following?



$[Co(NH_3)_5(OH_2)]^{3+}$ $[Co(NH_3)_5Cl]^{2+}$ $[Co(NH_3)_6]^{2+}$

   e. Using half-equations, workout a balanced equation for the formation of $[Co(NH_3)_5(OH_2)]^{3+}$ from $[Co(NH_3)_6]^{2+}$ by reaction with H_2O_2.
   f. Give a balanced equation for the synthesis of $[Co(NH_3)_5Cl]^{2+}$ from $[Co(NH_3)_5(OH_2)]^{3+}$ ignoring the counter anions.

13A.3 Risk assessment

A risk assessment for this experiment should have been prepared as part of the pre-laboratory exercises, and it should have been written neatly or printed and stuck in to the lab notebook before arrival at the laboratory session. This will be checked by a demonstrator before work can begin in the laboratory.

13A.4 Laboratory activity

Show a demonstrator the prepared lab notebook to confirm completion of the pre-lab exercises. Work in pairs but keep individual records in lab notebooks as the experiment proceeds. Choose a work area with a free locker and a fume cupboard to work in, and write the number of these at the top of the lab notebook page for this experiment. **Inform the demonstrator of the locker and fume cupboard number.**

Work in a fume cupboard for the duration of the experiment. Think carefully about use of stoppers and bungs to prevent flammable vapours escaping into the laboratory during any transfer of liquids during the experiment.

Part 1: Preparation of $[Co(NH_3)_5Cl]Cl_2$

The product from Part 1 will be used to prepare the nitro and nitrito complexes. Inform a demonstrator if the yield is less than 5.5 g. It is essential that the procedure is followed carefully if good yields are to be obtained.

1. Work in pairs in a fume cupboard. Dissolve ammonium chloride (5.0 g) in concentrated 0.88 ammonia (30 cm^3) in a 250 cm^3 conical flask. Continually agitate this solution whilst adding cobalt(II) chloride hexahydrate ($CoCl_2.6H_2O$) (10.0 g) in 2-3 g portions, making sure that each portion reacts before the next portion is added. Initially a precipitate of $[Co(NH_3)_6]Cl_2$ will form with the evolution of heat.

2. To the warm slurry/solution add, with care, 30% hydrogen peroxide (8 cm^3) in small portions (with efficient stirring). This results in a vigorous exothermic reaction with effervescence. A deep red solution of $[Co(NH_3)_5(OH_2)]^{3+}$ should form (sometimes this may look brown, which is not a problem).

3. Cool in an ice bath, then slowly add concentrated hydrochloric acid (30 cm^3). Heat the reaction mixture gently with stirring until a purple product precipitates (typically after 20-30 minutes) from a blue-green supernatant liquid. Do not allow the solution to boil. When the reaction is complete the supernatant liquid should be deep blue (to see this, let the mixture settle for a few seconds).

4. Cool to ambient temperature, and filter to obtain the solid product. Wash with several portions of ice cold water, then with a small quantity of acetone before drying in the air. The product should be a dry powder. If it is still wet, re-wash the sample with acetone on Buchner filter until free from water. Do not proceed with a wet solid. Record the mass of product obtained. **Show the product to a demonstrator.**

Part 2: Preparation of $[Co(NH_3)_5NO_2]Cl_2$, the nitro-isomer

1. Working in a fumehood, dissolve $[Co(NH_3)_5Cl]Cl_2$ (2.5 g) in a mixture of concentrated (0.88) ammonia solution (6 cm^3) and water (50 cm^3) by heating at the boiling point. Rapidly filter the hot solution through a Buchner funnel, cool the filtrate in an ice bath and acidify slightly to about pH 6 (check with indicator paper using a glass rod and white tile) by the addition of dilute hydrochloric acid.

2. Add sodium nitrite (3.0 g) to the cold solution, and heat the resultant mixture until the red precipitate that initially forms completely redissolves. Continue heating until the solution is dark yellow-brown in colour.

3. Cool the dark yellow-brown solution and then add concentrated hydrochloric acid (15 cm^3). Cool in ice for at least half an hour. Collect the brown-yellow crystals by filtration. Wash the product with a small quantity of acetone, then dry in the air. Record the mass of the product and determine the percentage yield (based on the amount of $[Co(NH_3)_5Cl]Cl_2$ used). Give a balanced equation for the formation of the product. **Place the product in an appropriately labelled sample bag and hand in to a demonstrator for marking**. If completing Experiment 13B next session, notify the demonstrator so that the sample can be set aside for use next session.

Part 3: Preparation of $[Co(NH_3)_5ONO]Cl_2$, the nitrito-isomer

1. Dissolve $[Co(NH_3)_5Cl]Cl_2$ (2.5 g) in a mixture of water (50 cm^3) and conc. (0.88) ammonia (6 cm^3) with heating as in Part 2. Filter the resultant solution, cool in ice then neutralise the filtrate with dilute hydrochloric acid. This step is absolutely critical to the success of the preparation. Use pH paper, a white tile and a glass rod to test the solution. A final pH of 6 can be tolerated.

2. Add sodium nitrite (2.5 g) and then 2.5 cm^3 of a 1:1 mixture of water and concentrated hydrochloric acid (prepare this solution in advance). Cool in ice for at least half an hour, then filter the red precipitate that gradually forms.

3. Wash the product with ice-cold water and a small volume of acetone before drying in the air. Record the mass of the product and determine the percentage yield (based on the amount of $[Co(NH_3)_5Cl]Cl_2$ used). Write the balanced equation for the reaction of $NaNO_2$ with concentrated HCl and thus construct a balanced equation for the formation of $[Co(NH_3)_5ONO]^{2+}$ from $[Co(NH_3)_5Cl]^{2+}$. **Show the lab notebook to the demonstrator for marking. Place the product in an appropriately labelled sample bag and hand in to a demonstrator for marking.** If completing Experiment 13B next session, notify the demonstrator so that the sample can be set aside for use next session.

Ensure that the lab notebook has been checked, samples have been handed in and apparatus has been washed up and put away. Tidy all work areas. **Have the work areas checked by a demonstrator before you leave the laboratory.**

DISCOVERY
BLOCK 4
EXPERIMENT 13B

ISOMERS OF COBALT(II) AND COBALT(III) COMPLEXES

13B. Isomers of cobalt(II) and cobalt(III) complexes

In Experiment 13A, linkage isomers of a cobalt(II) complex were prepared using an ambidentate ligand. In this experiment, these complexes will be investigated further, and a complex of cobalt(III) and a bidentate ligand will be prepared. The isomerism in these complexes will be investigated.

Recall that there are two main types of isomerism: structural (consititutional) isomerism (e.g. linkage isomerism and many others) and stereoisomerism (e.g. geometric isomerism and optical isomerism). This experiment will investigate examples of both constitutional isomerism (in this case, linkage isomerism) and stereoisomerism.

The complex to be prepared in this experiment contains various ligands that are capable of bonding to the cobalt(III) ion, namely: ethylenediamine (1,2-diaminoethane, $H_2NCH_2CH_2NH_2$, often abbreviated to en), water and chloride ions. By varying the number of these three ligands attached to the 6-coordinate metal ion, various isomers are theoretically possible.

In this experiment the complex ion $[Co(en)_2Cl_2]^+$ is prepared, which itself is capable of existing in isomeric forms, but here we are concerned with geometrical isomerism arising from the arrangement of the ligands about the metal ion. The cation has an octahedral co-ordination, so the two ethylenediamine molecules and two chloride ions can be arranged in two ways.

The formulation of the complex, i.e. $[Co(en)_2Cl_2]Cl$, has been written so as to indicate the presence of two types of chloride ions; one type is directly bonded to the metal, whereas the other is the counter-ion of the complex cation and is not directly bound to the metal. When the complex is dissolved in water the ions present in the solid lattice, $[Co(en)_2Cl_2]^+$ and Cl^-, become dispersed into solution; thus only one third of the chloride in the material is released as chloride ions.

In the preparation described below, the chloride counter-ion interacts with the excess hydrochloric acid used in the reaction to form the $[HCl_2]^-$ ion. Other ions of this type are well known, particularly $[HF_2]^-$.

13B.1 Aims

- To consider isomerism in transition metal complexes
- To prepare geometrical isomers of a cobalt(III) complex
- To prepare the nitro-isomer of a cobalt(II) complex by conversion of the nitrito-isomer

13B.2 Pre-lab exercises

These exercises must be completed at least one hour before the timetabled start time of the laboratory session. Students not completing the pre-laboratory task will be turned away from the laboratory until the exercises are completed. If desired, additional questions posed in the laboratory manual can be answered in the lab notebook before the session to ensure efficient use of time during the session.

1. Read the instructions in the laboratory manual through carefully and highlight unfamiliar words or apparatus. Use text books, the internet, LabSkills or the Interactive Lab Primer to look up the meanings of these unfamiliar terms.

2. Prepare a risk assessment for the experiment in the same way as performed previously in Experiment 10A. Use previous risk assessment tables that have been provided in laboratory manuals as a template. The table should list all the chemicals encountered in the experiment (including solvents, starting materials and products), the R and S (Risk and Safety) numbers associated with that compound and the R and S phrases written out in full. Use the MSDS (Material Safety Data Sheet) documents to identify the appropriate R and S numbers. Some have been provided on DUO. Others may need to be searched for on the internet. Cross reference these R and S numbers with the lists of phrases provided in DUO and copy them out in full into the table. Note that the R and S numbers are not normally given in your laboratory manual, but they should be included in your own risk assessments.

3. Ensure you possess a model kit and bring it with you to the laboratory session. Model kits are available to purchase from chemistry stores.

13B.3 Risk assessment

A risk assessment for this experiment should have been prepared as part of the pre-laboratory exercises, and it should have been written neatly or printed and stuck in to the lab notebook before arrival at the laboratory session. This will be checked by a demonstrator before work can begin in the laboratory.

13B.4 Laboratory activity

Show a demonstrator the prepared lab notebook to confirm completion of the pre-lab exercises. Work in pairs but keep individual records in lab notebooks as the experiment proceeds. Choose a work area with a free locker and a fume cupboard to work in, and write the number of these at the top of the lab notebook page for this experiment. **Inform the demonstrator of the locker and fume cupboard number.**

Model building work should be carried out on a clean bench away from any synthetic work. All synthetic work should be carried out in a fume cupboard. Ensure that appropriate balances are used, that all exact masses are recorded and that observations are recorded in the lab notebook throughout the experiment.

Part 1: Conversion of a nitrito-isomer of cobalt, $[Co(NH_3)_5ONO]Cl_2$, into the nitro-isomer $[Co(NH_3)_5NO_2]Cl_2$

Both isomers of this cobalt(II) complex were prepared during Experiment 13A. The nitrito-isomer slowly reverts to the more stable nitro-isomer at room temperature in the solid state, but the process can be accelerated by heating or by dissolution in acid solution. Retrieve the samples from Experiment 13A and compare. Note that it is pointless undertaking the experimental work if the product from Part 3 of Experiment 13A is yellow-brown. If it is, then the nitrito-isomer has already isomerised to the nitro-form. If samples belonging to both partners are yellow-brown, consult a demonstrator.

1. Dissolve about 0.5 g of the nitrito-complex prepared in Part 3 of Experiment 13A in hot water (5 cm^3) containing a few drops of dilute ammonia and cool the solution in an ice bath.

2. Carefully add concentrated hydrochloric acid (5 cm^3). Cool the solution thoroughly in an ice bath and isolate the yellow-brown crystals by filtration. Wash the product with a small volume of acetone and dry in the air.

3. Record the mass of product and calculate the % yield based on the amount of starting complex used.

4. Compare and contrast the two samples prepared in Experiment 13A with the product from this section. Name another ligand that is ambidentate (hint: look back through the lab notebook).

5. **Place the sample in an appropriately labelled sample bag and hand in.**

Part 2: Preparation of geometrical isomers cis- and trans-$[Co(en)_2Cl_2]Cl$

1. Add a 10% aqueous solution of ethylenediamine (1,2-diaminoethane) (15 cm^3) with stirring to a solution of $CoCl_2.6H_2O$ (4.0 g) in water (12.5 cm^3) contained in an evaporating dish or small beaker.

2. Add approximately 3.5 cm^3 of 30**%** hydrogen peroxide (the oxidant), adding quickly, but cautiously, in approximately 1 cm^3 portions.

3. Add a further 3.5 cm^3 of hydrogen peroxide dropwise. When the oxidation is complete, acidify the solution with concentrated hydrochloric acid (9 cm^3) and evaporate the solution slowly in a fume cupboard with continuous stirring at first, until a steady boiling rate is achieved. Use a hot plate and heat slowly. A crust forms over the surface as the volume is reduced (~ 20 cm^3). Do not leave unattended otherwise a paste containing concentrated hydrochloric acid will spatter.

4. The solution should then be cooled, and the bright-green square plates of the complex, *trans*-$[Co(en)_2Cl_2]Cl.HCl.2H_2O$ collected by filtration, and washed with a small volume of acetone and dried. The crystals should be green, not blue (which would indicate the presence of $Co(en)_3Cl_3$). If a visible mixture of blue and green coloured complexes is obtained, the blue complex may be removed by washing the solid with a small quantity of acetone. Consult a demonstrator before attempting this. Record the mass of the product and calculate the % yield based on the amount of $CoCl_2.6H_2O$ used. **Place the sample in an appropriately labelled sample bag and hand in to the demonstrator.**

5. The red solution contains the *cis*-isomer, but the latter is difficult to isolate without contamination with the *trans*-isomer. Discard the red solution in the heavy metals waste container located in the fumes cupboard.

6. Wash and put away all apparatus. Clean and tidy work spaces. **Have a demonstrator check the work spaces are clean and tidy.**

Part 3: Isomerism in cobalt(III) complexes

1. Draw the three isomers of $[Co(en)_2Cl_2]^+$ clearly into the lab notebook. Label each isomer with a term that differentiates it from the others. Assume that the geometry of 1,2-diaminoethane (en) restricts the positions of the nitrogen atoms to adjacent positions, i.e. cis-positions, and that en remains bidentate. Make models of the complex ions with your model kit, and take particular note of the features of the *cis*- isomer.

2. Draw all isomers of $[Co(en)_3]^{3+}$ (assuming en remains bidentate).

3. Suggest and outline an experiment that could be used to separate and isolate each of the different forms of $[Co(en)_3]^{3+}$. (Hint: refer to a text book such as Shriver & Atkins or Housecroft).

4. How many moles of AgCl would form upon addition of excess Ag^+ ions to an aqueous solution of $[Co(en)_2Cl_2]Cl$? (Hint: read back over the introductory text to the experiment).

5. **Show the lab notebook to the demonstrator for marking and sign out.**

APPENDIX A: Assessment guide for laboratory reports

Reports will be assessed against the following criteria, which are not necessarily equally weighted.

	Structure	**Presentation**	**Technical Content**	**Results and Discussion**
First Class	Excellent, very clear, logical subdivision.	Well written in good English, cogent arguments presented. Conclusions concur with results obtained, results are clearly summarised.	Appropriate theoretical background included. Proper use made of theory expressions, etc.	Critical assessment of the results. Quality of sample based on data (spectra, errors). Graphs neatly plotted and correctly interpreted. Extended interpretation based on analysis of theory section.
Upper Second	Well organised easy to follow and a sense of direction throughout.	Clearly laid out, conclusions and summary evident and clearly written.	Good grasp of the necessary theory and its use.	Results analysed and assessed in sufficiently critical manner. Evidence of an appreciation of sources of error.
Lower Second	Satisfactory but some loss of way evident.	Straightforward to read, satisfactorily written, vagueness or hesitancy in conclusions and summary.	Only the basic theory behind the experiment is presented, no evidence of real understanding.	Satisfactory assessment of results and outcome of experiments. Critical evaluation not overly evident.
Third	No direction, no subdivision. Lack of clarity.	Somewhat disorganised and hard to read. Conclusion and summary incorrect or “off the mark”.	Gaps in understanding evident from what was presented.	Poor analysis of the results or sample quality, no attempt to assess sources of error or where things may have gone wrong.
Fail	No evidence of any organisation, absence of basic understanding, no coherence.	Difficult to read; slap dash presentation, absence of conclusion or summary.	No real presentation of background and its appreciation.	No assessment of the results, no discussion.