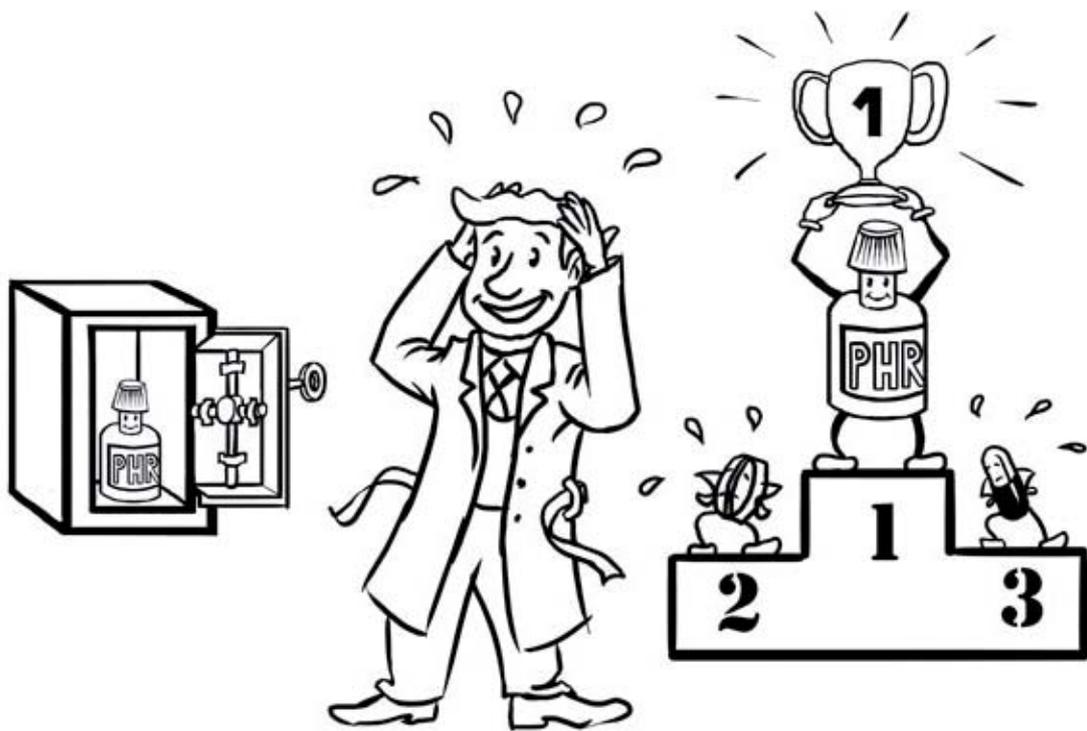


How are drugs developed?

Take part in the research of a drug



Experiment workshop
Student guide

➔ Introduction



Many stages are involved in the discovery and development of medicines. One of the most important stages is to find the molecule which acts as a drug and which has minimal side-effects.

Scientists from the Combinatorial Chemistry Platform of the Barcelona Science Park are investigating a new drug for the treatment of Parkinson's disease.

The treatment of Parkinson's disease currently consists of administering a cocktail of drugs to patients. However, in addition to the inconvenience of having to take various different drugs, this system of administration causes a high number of side effects.

The objective of this research group is to combine two drugs by means of a molecule chain, forming one single, more complex molecule which would make it possible to administer a single drug for the treatment of Parkinson's disease.

To carry out this research, we first need to obtain the drugs or molecules to be combined. To this end, the scientists from the Platform have set up a series of chemical reactions which has enabled them to obtain the desired product in the laboratory.

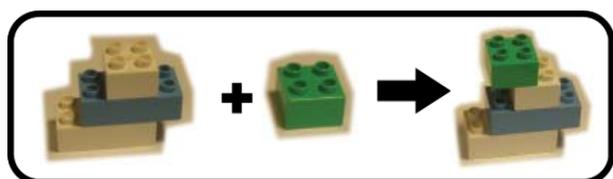


➔ Workshop objectives

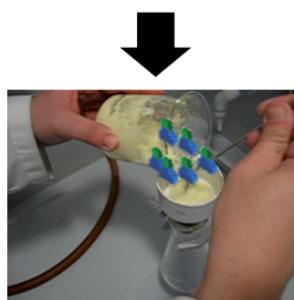
The objective of this experiment workshop is to become familiar with the research carried out in a chemical laboratory based on the search for new medicines. In particular, you will participate in the synthesis of one of the molecules being used for the research of a new drug for the treatment of Parkinson's disease.

➔ Organisation of the workshop

The workshop consists of three stages. First, a chemical reaction (1) will be conducted, which consists of making the pyrimidone react with the sodium nitrite. Secondly, the product (2) will be isolated by means of vacuum filtration, and lastly, the product obtained (3) will be characterised by thin-layer chromatography, using an ultraviolet lamp, to verify whether the desired product has been obtained.



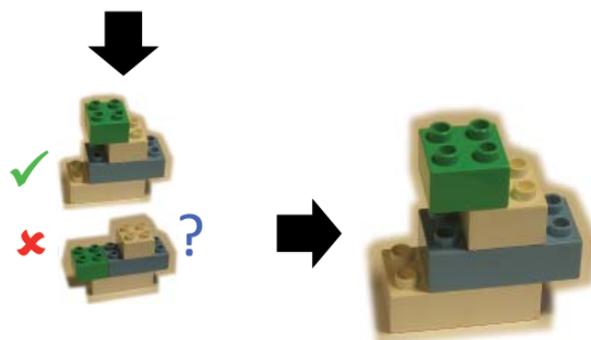
1. Chemical reaction



2. Isolation of the product through filtration



3. Characterisation of the product through chromatography



This chemical reaction is only a small part of the overall process, but it is crucial for obtaining the desired product.

Laboratory notes:

In the laboratory notebook, note down each stage that you carry out, as well as any changes that you observe. Also note down the results and the final conclusions, just like scientists do in their laboratories.

➔ Equipment and materials required

			
stand	clamp and clamp holder	round bottom flask	Kitasato flask
			
graduated cylinder	funnel	Büchner funnel	rubber adapter
			
spatula	weighing trays	Scissors and tweezers	capillary glass
			
magnetic stir bar	stopper	Filter paper	Eppendorf tubes (only to be used by the monitor)
			
beaker	silica layer	glass vial	wash bottle
			
balance	magnetic stirrer	vacuum pump	UV lamp
Reagents:			
			
Pyrimidone (toxic) *	Sodium nitrite (toxic) *	42% acetic acid solution (corrosive) *	Chromatography solvents (toxic) *

* Consult safety instructions for these chemical products

Other material needed : pencils, markers, lab coats, gloves, goggles and a residues container

➔ Safety Instructions

Be informed

Make sure you know the location of the safety equipment in the laboratory or areas set aside for experiments (fire extinguishers, showers or toilets, exits, etc.). Read the instructions carefully before conducting an experiment. Remember to read the safety labels on reagents and apparatus.

Wear suitable clothing

Gloves, lab coat and protective goggles.

General rules

Smoking, eating and drinking are not allowed in the laboratory or areas set aside for experiments. Always wash your hands before leaving the laboratory. Work in a clean, orderly, unhurried manner. If a product is spilled, clean it up immediately. Always leave the material clean and tidy. Never use a piece of equipment or apparatus without knowing exactly how it works.

Handling glass

Wear protective gloves when handling glass material and pay special attention to the temperature of said material, since hot glass is indistinguishable from cold glass. Never use cracked glass.

Chemical products

Never use an unlabelled bottle of reagent. Never smell, inhale, taste or touch chemical products. Do not pipette by mouth. If you are using toxic or corrosive products, always wear gloves and wash your hands often. Never place containers with reagents near a flame. Do not heat flammable liquids. Carry bottles by holding the base, never by the neck.

Waste disposal

Place in special, suitably labelled containers: broken glass, reagents which are toxic, noxious or harmful to the environment, and biological waste. Never pour solid waste down the drain.

In the event of an accident, notify the instructor immediately. - Remember: If you have any questions, ask the instructor.



Specific precautions for this workshop

In this practical session, you will handle chemical reagents and organic solvents with the following hazards:



Pyrimidone: Toxic if ingested or if in contact with the skin.



Sodium nitrite: Danger of fire if it comes into contact with combustible substances. Toxic if ingested or if in contact with the skin.



42% acetic acid : Acetic acid is corrosive; avoid contact with the skin and do not breathe in vapours.



Solvent A (ethyl acetate): Highly flammable. Toxic if inhaled, ingested or if in contact with the skin. Irritation eyes.



Solvent B (methanol): Toxic if inhaled, ingested or if in contact with the skin. Highly flammable.

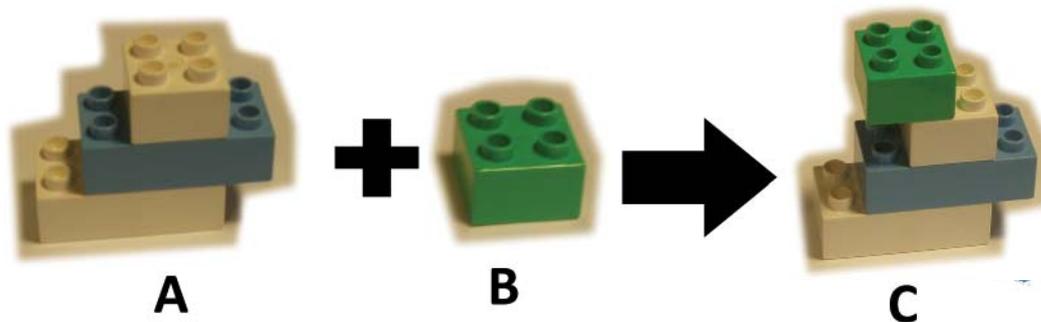
➔ Procedures

1. The chemical reaction

The synthesis of a drug comprises a number of stages. In each stage, a chemical reaction occurs which consists of a process through which one or more substances (reagents) undergo a transformation or combination to form different substances (products).

Chemical reactions are represented by chemical equations, which consist in graphically representing the process which is taking place.

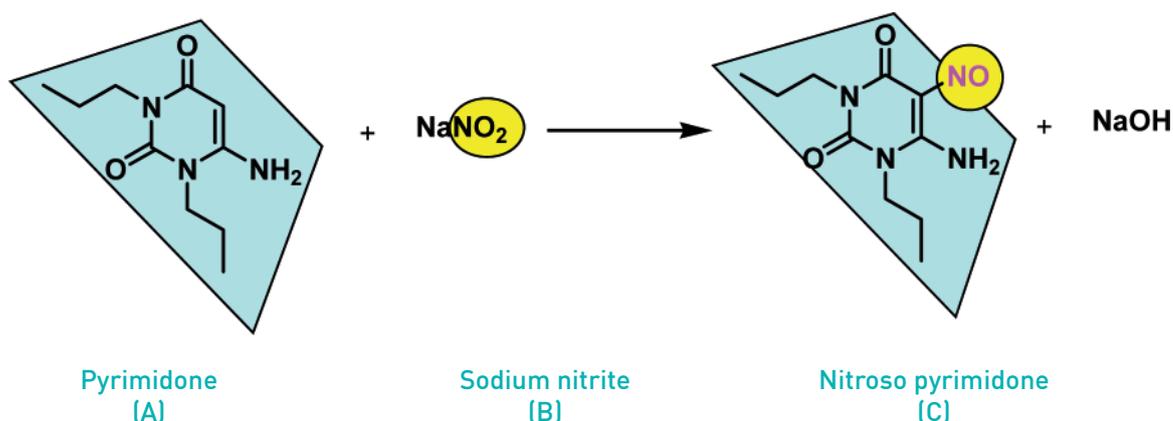
CHEMICAL EQUATION: $A + B \rightarrow C$



Example: Acetic anhydride + salicylic acid ->



In the chemical reaction that you will be conducting, the pyrimidone (A) will react with the sodium nitrite (B) to form the product nitroso pyrimidone (C)

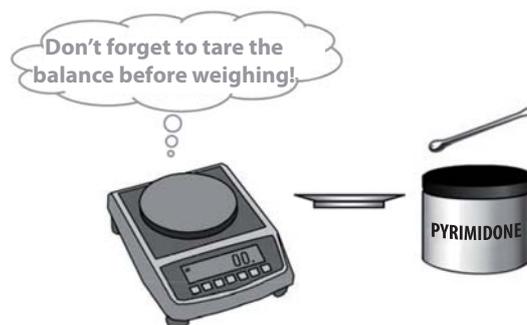


Protocol for the chemical reaction

- 1** Prepare the material for the reaction.

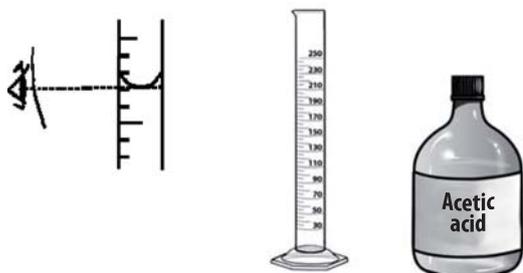


- 2** Weigh 1.0 g of the initial pyrimidone in a weighing tray and add it to the round bottom flask using the funnel.



- 3** Measure 50 ml of the 42% acetic acid aqueous solution, completing to volume as shown in the diagram.

CAUTION! DO NOT BREATHE IN THE VAPOURS!



- 4** Gradually add the acetic acid solution from the graduated cylinder to the flask containing the pyrimidone using the funnel, and turn on the magnetic stirrer.



- 5** In another weighing tray, weigh 0.49 g of sodium nitrite (NaNO_2) and gradually add it to the round bottom flask. You will immediately observe the appearance of a solid.



- 6** Leave the reaction stirring at room temperature for 10 to 15 minutes, with the round bottom flask covered.

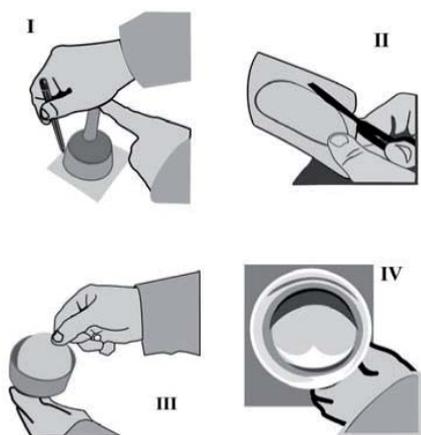


2. Isolation of the product through filtration

Vacuum filtration is a technique for separating solid-liquid mixtures. The mixture is introduced into a filter funnel with the filter paper attached to the base. The mixture is sucked out by means of a vacuum pump applied to the base of the funnel, leaving the solid trapped in the pores of the filter. The remainder of the mixture passes through the filter and is deposited on the bottom of the container. This technique is faster than normal gravity filtration and is recommended when such filter processes are very slow.

Protocol for isolating the product through filtration.

- 1** Draw and cut out the filter. Place it in the Büchner funnel, without letting it touch the funnel's sides.



- 2** Hold the KITASATO flask in place with the clamp, and set the rubber adapter and the Büchner funnel that contains the filter in place. Connect the KITASATO flask to the vacuum pump and moisten the filter with a few drops of water.



- 3** Carefully pour the contents of the flask into the Büchner funnel. Wash the solid thoroughly with distilled water and leave it to air dry (2 min)*.



- 4** Weigh the solid obtained in the vial and note down the weight.



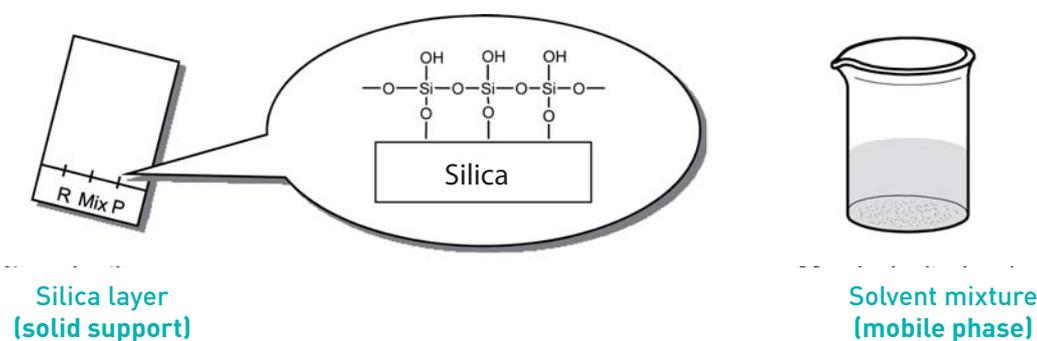
*NOTE: if the solid is very moist, ask the instructor to wash it with a few drops of acetone, and leave it to air dry for a little longer.

3. Characterisation of the the product through thin-layer chromatography

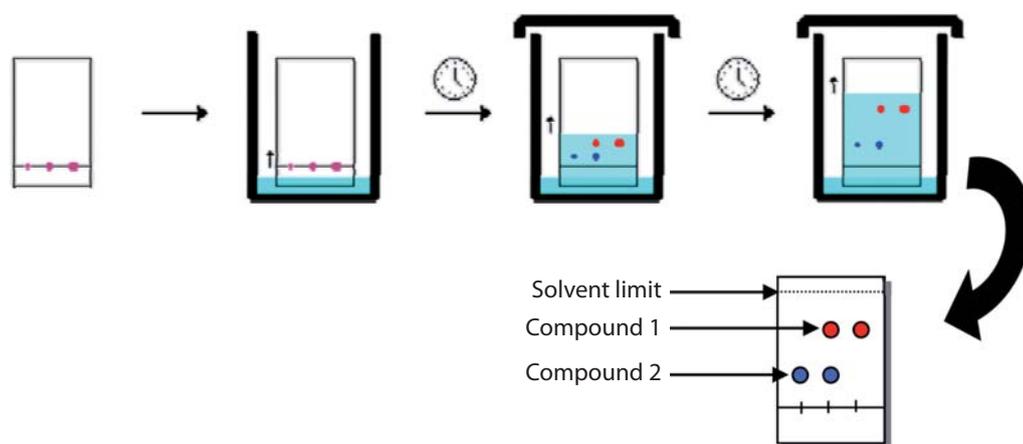
Thin-layer chromatography (TLC) is a separation technique frequently used in organic chemistry laboratories, as it enables us to:

- Compare samples
- Monitor a reaction
- Determine the purity of a compound

This technique uses a solid support (silica layer) and a mobile phase (comprising a solvent or a mixture of solvents)



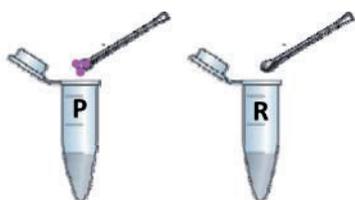
The test sample is applied over the silica layer, is absorbed by the same, and is introduced into the mobile phase. By means of capillary action, the mobile phase rises through the solid support which contains the sample components. Each compound has a different chemical nature and therefore a different affinity for both the solid support and for the solvent, which means that they will rise through the silica layer at different rates. Each stain in the silica layer will therefore correspond to a different compound.



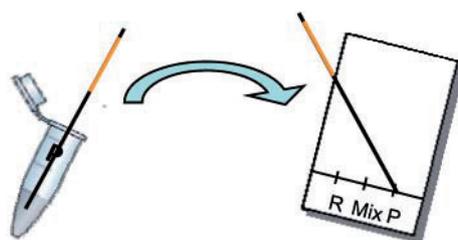
Using this technique, we will know whether the reaction had finished at the moment we stopped it, and it will also give us a quantitative idea of the degree of purity of the product that we have obtained.

Protocol for characterising the product through thin-layer chromatography

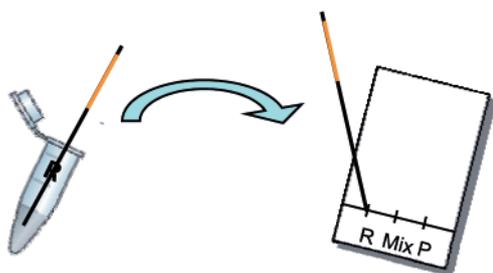
- 1** Dissolve a small amount of the product obtained (the tip of a spatula) with acetone in an Eppendorf tube. Do the same with the initial pyrimidone.



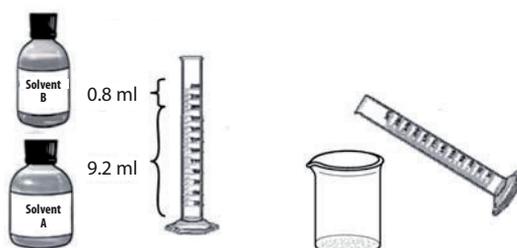
- 2** Introduce the capillary glass into the product solution. Then place the capillary glass on the stain in the thin-layer (P) and in the middle (Mix), ensuring that the stain is as small as possible.



- 3** Carry out the same operation with the initial pyrimidone (R and Mix).



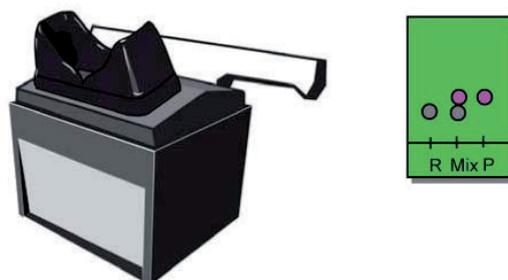
- 4** Prepare the mobile phase: in a 10-ml graduated cylinder, measure 9.2 ml of solvent A (Ethyl acetate) and 0.8 ml of solvent B (methanol). Pour the contents into the beaker, cover and stir.



- 5** Carefully introduce the thin layer into the beaker, cover it with the lid and allow the liquid to rise by means of capillary action. When the limit of the solvent is 0.5 cm from the upper part of the plate, remove it.



- 6** Observe the result of the thin-layer under ultraviolet light. Mark the stains that you identify with a pencil.



➔ Additional information

Websites on Parkinson's disease

Special edition of Nature: Outlook Parkinson Disease

<http://www.nature.com/nature/outlook/parkinsons/index.html>

Actor Michael J. Fox's foundation

<http://www.michaeljfox.org/index.cfm>

Videos and tutorials from the National Library of Medicine, USA

<http://www.nlm.nih.gov/medlineplus/ency/article/000755.htm>

<http://www.nlm.nih.gov/medlineplus/tutorials/parkinsonsdisease/htm/index.htm>

Websites on the techniques used in this workshop

Animated film on thin-layer chromatography

<http://www3.wooster.edu/chemistry/analytical/gc/default.html>

Others on chromatography

http://en.wikipedia.org/wiki/Thin_layer_chromatography

http://www.shsu.edu/~chm_tgc/sounds/flashfiles/GC.swf

http://en.wikipedia.org/wiki/Gas_chromatography

Animated films on membrane potential, synapses and neurotransmitters

<http://outreach.mcb.harvard.edu/animations/actionpotential.swf>

http://highered.mcgraw-hill.com/sites/0072437316/student_view0/chapter45/animations.html#

<http://www.celanphy.science.ru.nl/Bruce%20web/Flash%20Movies.htm>

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