Monitoring public opinion on Nanotechnology in Europe

European Platform on Nano Outreach and Dialogue (NODE)
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1. Executive Summary

Hands-on activities are one of the best tools to convey fundamental and applied concepts of nanotechnologies, especially to youngsters, who regard these as the most engaging tools. This was reported by many students and teachers that participated in previous outreach projects, where schools were provided with several experiments that could be done in school laboratories. During the evaluation phase of these projects, some ideas and recommendations were collected for the development of future experiments; this valuable input has been used in the development of two "NANOPINION Experiments" which are reported in this document, and which constitute Task 3.5 of the project. The aim of this task is to produce two simple, hands-on laboratory experiments that can be performed in a teaching laboratory (for schools and science centres). Teacher documentation and student laboratory worksheets are also prepared for each experiment, the latter written using an enquiry-based approach. A third experiment was developed, which is a "virtual experiment", meaning an experiment that can be done online using a "virtual laboratory". This experiment was designed with in mind the request of students (collected in previous projects) to "move atoms" using a real Scanning Tunnelling Microscope. Since the actual remote usage of a real STM is not possible for a large international audience, the NANOPINION virtual experiment was developed to provide students with this opportunity, virtually using the instrument not just to "move atoms" but also to simulate a "real" experiment where the instrument is used to create nano-sized molecular transistors.

2. INTRODUCTION

There are numerous reports that emphasise the need to "revitalise" science teaching in school, particularly at the high-school (14+) level. It is also often recommended in those reports to encourage inquiry-based science education (or problem-based learning), where teaching is conducted through an inductive (rather than deductive) method. This should be combined with numerous "hands-on" activities to allow students to see science for themselves, and then learn and understand the theoretical explanation of what they see. Nanoscience and nanotechnologies offer such opportunities. Nanoscience and nanotechnologies offer teachers a new instrument to bring exciting science and technology into the classroom. Nanotechnologies are now used in numerous devices young students are very familiar with, such as computers, mobile phones and iPods. Nanoscience offers the possibility to improve numerous material properties and create new ones; in the future we will have more and more products that incorporate some form of "nano"-either a nanomaterial, or a nano-enabled technology. Bringing "nano" into the classroom means bringing in the latest cutting-edge science and technology and talking about very exciting future scientific developments.

2.1. The importance of "hands-on" activities

One of the peculiarities of nanoscience is that numerous "nano-effects" can be seen in our "macro world". The best example is a gold colloid (made of gold nanoparticles about 15 nm dispersed in water) which is red in colour. When some salt solution is added to the gold colloid, it turns blue! There are many "hands-on" activities and demonstrations that can be used to show the properties of nanomaterials – effects that are visible! So even though the "nano-world" is invisible, we can appreciate its effects in materials that youngsters are very familiar with, like gold.

Previous EC-funded projects, like Nanoyou[1] and Time for Nano[2], have demonstrated the usefulness of hands-on activities for engaging young people as well as the general public in nanoscience. Simple experiments can be used to demonstrate basic nanoscience principles, which can then be further discussed in outreach activities using end-products, like commercial shirts and creams, this way linking nanoscience theory with practical applications.

2.1.1. Nanoscience school experiments

Nanoscience is often referred to as a converging science in the sense that it derives from the convergence of "traditional" disciplines like chemistry, physics and molecular biology. By its nature, nanoscience is interdisciplinary and teachers (and students) involved in this field need to be able to cross the boundaries of their specialization. This opens new challenges in terms of teachers' collaboration, teaching methods and communication.

Simple experiments for school aged students have already been produced by several educational specialists and university; these experiments are interdisciplinary by definition, and have been shown to be very useful to illustrate simple nanoscience concepts (e.g., that the colour of a metal depends on its nanosize), and to illustrate in practical terms the concept of "interdisciplinarity". Table 1 lists some international organizations (outside the EU) that have produced nanoscience school materials, which include teaching reading resources and experiments.

PROJECT NAME	DESCRIPTION	FUNDING BODY
Nanosense project, www.nanosense.org:	This is probably the biggest project oriented at making training materials for teachers. There are some very good PDFs free to download that cover aspects such as, teaching nanoscience in school, ideas on how to integrate nanoscience in a chemistry or physic curricula, as well as background documents, PPTs, exercises etc.	This project (ended) was founded by the National Science Foundation (NSF)
MRSEC Education group http://mrsec.wisc.edu/Edetc/:	The biggest educational portal in terms of material, lesson plans, lab exercises, etc. made by scientists together with experts of communication precisely about nanoscience. It had some material for teachers.	This is an educational portal of the University of Wisconsin-Medison, with funding also from NSF.
Nanoscience Informal Education Network (NISE network), www.nisenet.org	This is one of the best network of informal educators and it has material for schools, as well as science exhibits, nano-days etc. Members of the network can share ideas etc. Some of the material has been reviewed by the teachers and their comments are reported. Some experiments are a simplified version of the ones developed by the Wisconsin-Madison University (above). Many simple	Funded by the NSF.

[1] www.nanoyou.eu
[2] www.timefornano.eu

	demonstrations for young children and general public.	
AccessNano, www.accessnano.org	This is an Australian educational resource for secondary schools. Lots of material and very well done. It has an industry-application focus.	AccessNano is an Australian government initiative funded through the Australian Office of Nanotechnology, under the Department of Innovation, Industry, Science and Research in working with the Department of Education, Employment and Workplace Relations. AccessNano has been produced by foresight and science communications consultancy Bridge8 Pty Ltd. The material was developed in close collaboration with industry, academia and science teachers across Australia. Partners are also Nanotechnology Victoria (www.nanovic.com.au). Nanotechnology Victoria Ltd (Nanovic) is a venture for delivering nanotechnology research outcomes to Victorian industry. It is a venture of 3 major universities, and also receives funding from the Australian Government.
Nano Education Portal : http://www.nanoed.org/,	This is a big web educational portal with educational resources, news, seminars etc. on teaching nano at school (all levels).	Funded by NSF.

Table 1 International organizations (outside the EU) that have produced nanoscience school materials.

The importance of developing educational tools on nanotechnologies has been recognized also in the EC and during the 7th Framework Project this vision resulted in various FP7 projects specifically funded to create and test outreach and educational resources for teachers, students and lay public. The next table[3], Table 2, lists some projects in the EU and Associate Member States that have developed teaching resources, including experiments, on nanoscience for school-aged students and their teachers[4].

PROJECT NAME	TYPE OF RESOURCES DEVELOPED
DG RESEARCH	Video
NANOYOU	Hands-on activities; Experiments; Power point presentations; Reading material; Videos; Posters; Virtual lab; Virtual games; Games; Dialogue activity kit; teacher training kit
TIME FOR NANO	Hands-on activity kit; Dialogue activity kit; Videos
NANO-TV	Videos
NANOCHANNELS	Podcasts; Videos; Reading materials
OBSERVATORY NANO	Virtual game
NANOCAP	Reading material

Table 2 List of projects in the EU and Associate Member States that have developed teaching resources, including experiments, on nanoscience for school-aged students .

Appendix I lists some commercial or free kits that have been developed containing simple games, demonstrations and hands-on activities to be used in schools and science centres to illustrate fundamental concepts of nanoscience and applications of nanotechnologies.

2.2. Virtual experiments- a valuable tool in nanoscience education

Virtual experiments can be extremely valuable to bring students closer to emerging sciences, like nanoscience, which make use of instruments that are impossible to bring to school, such as some very sophisticated microscopes. Virtual experiments are therefore a success when they provide students with a learning experience that could not be provided to them otherwise. They should therefore not be seen, nor be used, as an alternative to hands-on experiments, rather as a complementary tool.

Previous EC FP7 projects, like NANOYOU or XploraHealth[5] have developed such tools and found that they attract student interest because they provide a format (use of an online, interactive "learning game") they can easily use and relate to. Teachers on the other hand sometimes report having some difficulties using online virtual experiments since they are a new educational

[3] Compiled using Nanopinion D1.1 and D3.1

[4] For a comprehensive list of projects that have developed outreach and communication tools in nanotechnologies, for different stakeholders, including teachers and students, see Nanopinion D1.1 "Content mapping review and exploitation strategy of past FP6/7 and OECD results", 2012.

[5] http://www.xplorehealth.eu/en

format they have less experience with. The success of virtual experiments is therefore often the result of a combination of factors: the relevance of the experiment to student interests and the confidence the teacher has in using this innovative tool.

2.3. Impact and recommendations from previous educational projects

Among the many resources that were developed and tested, experiments and hands-on activities were recognised as one of the best tool to engage youngsters and lay public on nanotechnology, to teach some fundamental nanoscience principles, and to show some peculiar properties of products developed using nanotechnology. The NANOYOU project in particular provided a group of 20 pilot schools with a large selection of tools (posters, video, virtual activities, hands-on experiments, etc.). Students and teachers were asked to test those resources in class, and provide feedback through an online questionnaire. Figure 1 and Figure 2 show some results of the evaluation done at the end of the NANOYOU project on the tools schools tested. The tools that were preferred by the students were hands-on activities and the video (Figure 1), and all the experiments (a total of four were provided) were assed as being "very good" by the majority of the students. Interestingly, the experiment that had the lowest level of appreciation, experiment C, was also the experiment that required the most expensive consumables, and that had a more theoretical (rather than application-based) approach (more similar to a "classical" chemistry experiment).

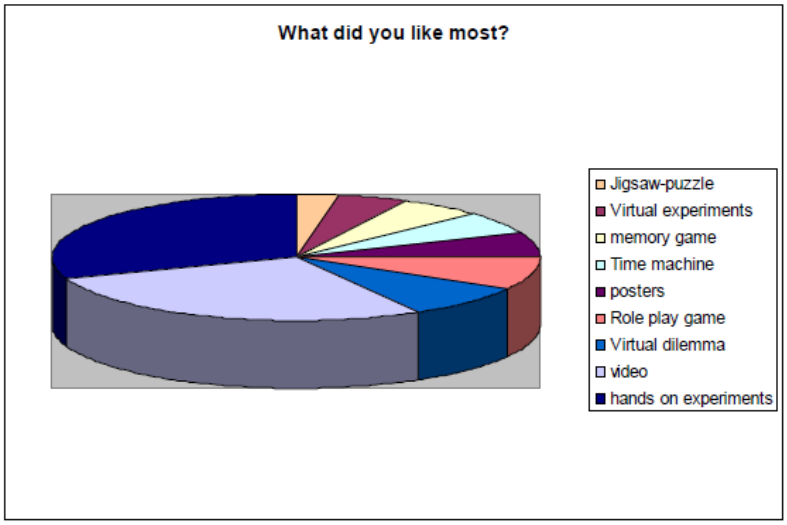

Figure 1 Assessment of NANOYOU materials (1), source: NANOYOU student survey 2011.

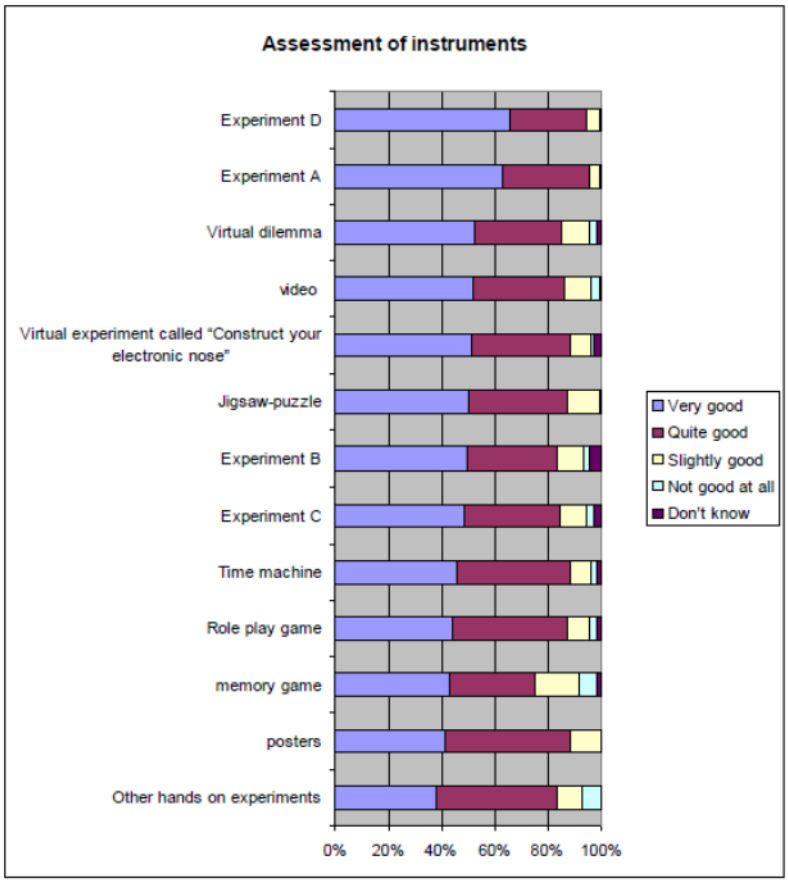

Figure 2 Assessment of NANOYOU materials (2), source: NANOYOU student survey 2011

During the NANOYOU project many comments were collected from the teachers of the pilot schools participating in the project, during the training events (through formal questionnaires), during live events (through observation and interviews after the event), and through some informal communication channels, like the shared Basecamp platform. Overall, the following **recommendations and ideas for future material development were collected**:

1) Develop more knowledge and pedagogical based-resources.

HANDS-ON NANOSCIENCE:

- Offering more practical and demonstration materials:
- Have a larger number of accessible practical experiments.
- Make it simple & fun
- Focus on "visible" nano-effects
- Enquiry-based approach
- Use real lab images

FOCUS ON APPLICATIONS:

- Relate to students needs/interests
- Analysis of commercial products

2) Develop more computer based materials

VIRTUAL SIMULATIONS:
- Moving atoms- (e.g., develop a "virtual STM")
- Virtual experiments of real nanolab experiments
- Zoom-in simulations

ONLINE LEARNING PLATFORMS
- Offering more videos made by experts in research
- Offering an online platform where students could ask scientists questions in regards to nanotechnologies
- Increasing the computer-game approach in the games offered such as the memory games and puzzles

All those recommendations were taken into consideration when planning the educational tools development of the NANOPINION project, which include new hands-on experiments, one new virtual experiment, two videos, a totally new e-learning mini-course, and a discussion game[6].

3. NANOPINION NT LAB EXPERIMENTS

This chapter describes with some details the new experiments developed by the NANOPINION project, two of which are "hands-on" and one is "virtual". The full teacher and student documents are provided as Appendix II, III and IV. A pedagogical guide on how to use these experiments in class, together with the other educational tools developed by the NANOPINION project, is provided as a separate document called "NANOPINION Teacher Guide" which is available on the project website.

3.1. HANDS-ON- EXPERIMENT A

3.1.1. Aim

The aim of this experiment is to illustrate through a simple model how a miniaturised drug delivery system is created and how the release of the drug herein contained can be controlled. The drug delivery system is created through a self-assembly encapsulation method, by using a natural polymer (alginate) and a food dye, which in the model represents the drug. In the experiment students can learn how the release of the "drug" depends from a number of variables, like the type of the drug encapsulated (this is tested by changing the food dye) and the media where the nanocapsules are released in. In addition, students test the effect of adding a "shell" to the nanocapsule to stop or slow down the release of the drug. In this experiment the "shell" is a nanocoating of chitosan, another natural polysaccharide. The coated nanoshells are also tested in different media, like water and milk (here used as a model for a complex biological media). Overall the experiment is a simple way of discussing the many variables involved in developing nanoscale drug delivery systems, and the use of nanoshells to control the delivery.

The full teacher and student document is provided in Appendix II and III.

[6] All NANOPINION educational tolls are available at www.nanopinion.eu under the section "Education" (http://www.nanopinion.eu/en/education)

3.1.2. Teaching goals

- Familiarize students with the concept of self-assembly as a method to create nanomaterials, in particular nanocapsules, and the forces that are involved in this process;
- Discuss the variables that scientists need to consider when designing a drug delivery system;
- Study how some variables, like drug composition (chemical structure, solubility, charge, etc.), nature of the capsule and its outer shell, and media, influence the diffusion of the "drug" from the drug delivery system;

- Discuss the effect of the "shell" and how a core-shell system can be used to control drug delivery, either passively or actively;

3.1.3. Summary of Experiment

In the first part of the experiment students will learn a simple way of making "capsules" that in the second part of the experiment will be used as "drug carriers". The capsules form through a cross-linking process that occurs spontaneously when mixing a solution of alginate salt and calcium chloride. A regular, spherical structure is formed (in this document called "bead"), hence this is a simple example of self-assembly. The capsules formed are not nanosized but rather micron-sized. However, the same material is used by scientists to create nanocapsules.

In the second part of the experiment, students will encapsulate a "drug" inside the calcium-alginate capsules. This process is done during the self-assembly step, since the "drug" (in this model represented by a food dye) is added to the alginate salt solution. The alginate mixture (having a strong colour) in now added drop-wise to the CaCl2 solution, as described previously. Coloured beads are formed. Two systems are compared, one using a red dye and one using a blue dye.

Figure 3 (Left): Calcium alginate beads with a red food dye entrapped; (middle): Red calcium alginate beads in water after overnight immersion; (right): Calcium alginate beads with a blue food dye entrapped.
Image credit: L. Filipponi, iNANO, Aarhus University, Creative Commons Share Alike Non-Commercial 3.0.

The dye is trapped inside the calcium alginate beads, and its diffusion in water and milk is compared. Students should make some hypothesis on the fact that this process depends on the type of media the beads are placed in and on the type of the dye used. In this experiment the milk is used to simulate a biological fluid (contains proteins, minerals, fat molecules), therefore a much more complex media than water.

Figure 4(Left): Red calcium alginate beads in milk after overnight immersion; (Middle): comparison between beads after overnight immersion in milk, surnatant, and control (right vial, contains only milk); (Right): Red calcium beads after overnight immersion in water (left vial) and in milk (right vial); Inset shows the a close view of these same beads (left bead: overnight immersion in water; right bead: overnight immersion in milk).
Image credit: L. Filipponi, iNANO, Aarhus University, Creative Commons Share Alike Non-Commercial 3.0.

Two systems are compared, one using a red dye and one using a blue dye. The two food dyes differ for chemical structure and charge. The blue dye diffuses rapidly from the bead (after 15 minutes release is observed) whereas the red dye is trapped strongly.
In the third part of the experiments, students add a "shell" to the beads and study how this affects the release of the food dye. The shell is made of chitosan, a natural polysaccharide which come from the shell of crabs and other crustaceous.

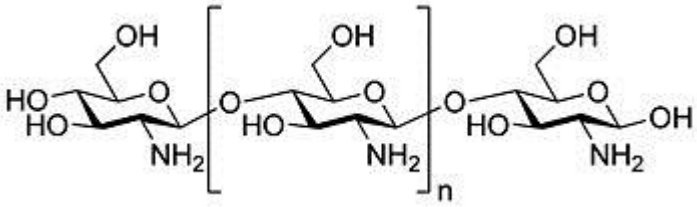

Figure 5 Chemical structure of chitosan

Students will observe that adding an outer nanocaoting to the calcium alginate bead strongly influences the dye release. This effect is seen when using both the red and the blue dye, but it is most notable in the latter case.

The dye is trapped inside the calcium alginate beads, and its diffusion in water and milk is compared.

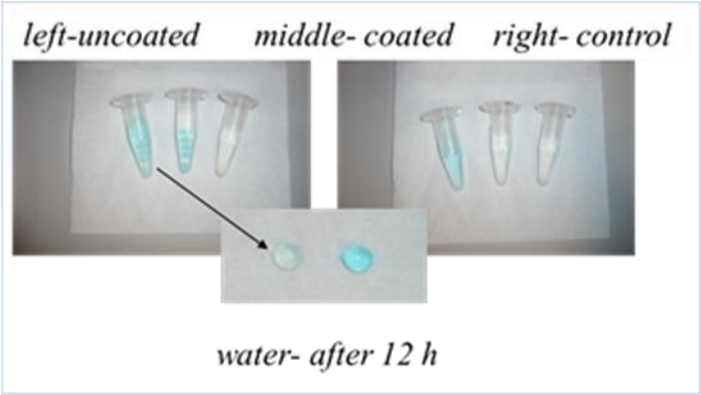

Figure 6 The effect of the "chitosan shell" on the release of the blue dye from calcium alginate beads in water. After 12 hours (overnight test) the coated beads still retain the blue dye and minor release is observed. As indicated in the figure, the vial on the left contains uncoated beads, the one in the middle coated beads, and the one on the right water as control.
Image credit: L. Filipponi, iNANO, Aarhus University, Creative Commons Share Alike Non-Commercial 3.0.

3.1.4. What does the experiment teach/show about nanoscience

The general concept behind many novel drug delivery systems is to "trap" the drug inside a capsule, which acts as a carrier to deliver the drug at the target, in some cases passing through the cell barrier and reaching the inside of the infected cell. The capsule has a protective shell that prevents the capsule from being dissolved before reaching the target. In addition, some biomolecules can be attached to the outer shell, which are specific to the target.
Figure 7 provides a schematic overview of encapsulation technology used for drug delivery.

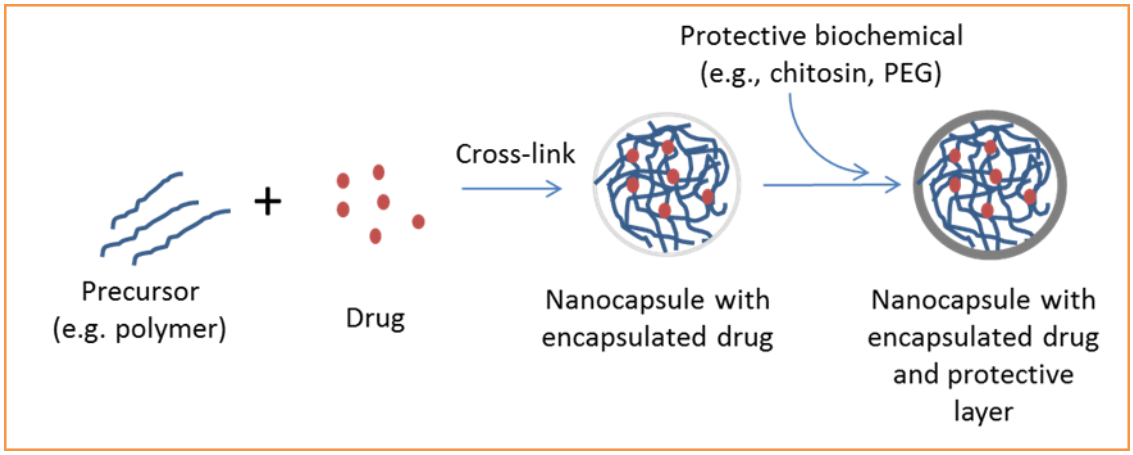

Figure 7 Overview of encapsulation technology.
Image credit: L. Filipponi, iNANO, Aarhus University, Creative Commons Share Alike Non-Commercial 3.0.

In the example illustrated in Figure 7 the nanocapsule is formed by self-assembly, using a material, for instance a polymer that cross-links in certain conditions and entraps the drug during the self-assembly process. The protective layer (often called a "shell") can act as an anti-fouling agent; can have specific target molecules that identify and reach the target; can have specific solubility properties, i.e., be degraded by enzymes that are located only at the target site or be stable only in specific acidic conditions.

The present experiment is a model of a drug delivery system. By "*model*" it is meant:

- The nanocapsule is represented by a macroscopic sphere (referred as "bead") which is made of a naturally occurring polymer, alginate;
- Like for many nanocapsules developed in research, the alginate bead is formed by self-assembly;
- The drug is simulated by a dye, which gets entrapped in the bead during the self-assembly process;
- The release of the drug (i.e., the dye) is studied in two media, water and milk. Milk simulates a biological fluid, since milk contains many different biomolecules and enzymes;
- The control of the release is simulated by placing a nanocoating on the outer surface of the bead. The nanocoating is made of chitosan, another natural occurring polymer. The resulting system simulates a "core-shell" system, where the "shell" acts as a protective layer, and the "core" carries the drug;

Hence overall the experiment is a simple way of discussing the many variables involved in developing nanoscale drug delivery systems, and the use of nanoshells to control the delivery.

3.2. HANDS-ON: EXPERIMENT B

3.2.1. Aim

Thin films with nanoscale thickness are interesting novel materials that are being investigated in "smart" windows (for instance electrochromic and thermochromic thin films) and in biosensors (for medical detection, food monitoring, etc.). In this experiment students will produce electrochromic thin films of Prussian Blue having different thickness through electrodeposition. To perform the synthesis, they will build a graphite counter electrode. A simple extra laboratory activity (which can be done as a separate, free-standing laboratory activity) is suggested to further demonstrate that this nanomaterial is conductive, as oppose to other allotropes of carbon like diamond. This activity can be used to illustrate a fundamental concept in nanoscience: the nanostructure of a material can affect its properties in unique ways. In the second part of the experiment, the students study the optical properties of the thin films (absorbance) and verify a fundamental law in optics, the dependence between film thickness and magnitude of the absorbance. In the third part of the experiment the students verify the well-known electrochromic properties of Prussian blue thin films.

The full teacher document is provided in Appendix IV.

3.2.2. Teaching goals

- Teach students one of the methods for preparing nanoscale thin films;
- Thin film visible light absorbance;
- Prussian blue, a material with peculiar optical properties;
- Prussian Blue thin films: application to biosensors (medical, food, etc.);
- What is electrochromism, and how it can be used in real-life applications;
- Conductivity of graphite;

3.2.3. Summary of Experiment

The first part of the experiment consists in the preparation of a thin film with nanometre thickness. There are numerous methods that researchers use in their laboratories to prepare thin films, for instance spin coating, sputtering, and dip-coating. Under specific conditions, and using some specific instrumentation, these methods allow the formation of thin films with controlled morphology, chemistry and thickness. In a school laboratory those instruments are not readily available; therefore it is suggested to prepare the thin film by electrodeposition, using a simple electrochemistry cell designed for this experiment. The advantage of this method is that it allows obtaining thin films with controlled film thickness just by controlling the synthesis time. As such, electrochemistry is interdisciplinary; hence it combines fundamental aspects of chemistry (red-ox reactions, etc.) and physics (Ohm law, etc.).
In this experiment students will prepare nanoscale thin films of Prussian Blue, a well know electrochromic material.

In professional laboratories, an electrochemical cell like the one shown in Figure 8 (left) is normally used. However, these types of cells are custom-made and fairly expensive; therefore a cheaper alternative is suggested. Figure 8 (right) shows the materials needed and the cell once it is set up:

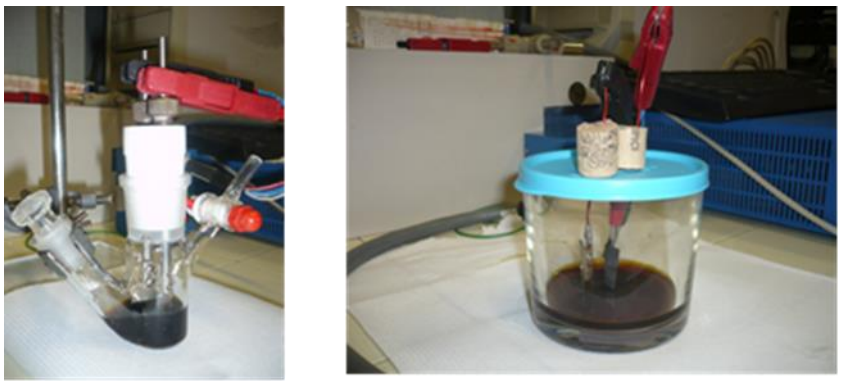

Figure 8 (Left): An electrochemical cell used in professional laboratories; (Right): a simplified electrochemical cell proposed in this protocol.
Image credit: L. Filipponi, iNANO, Aarhus University, Creative Commons Share Alike Non-Commercial 3.0.

The synthesis is normally done using a Pt counter electrode and ITO glass as a surface where to deposit the Prussian blue thin film; in the protocol some cheaper alternatives are provided, namely a graphite counter electrode and a plastic-coated ITO working electrode.

In addition, the synthesis needs an instrument to provide a constant current flow to the cell; this instrument is called galvanostat. The author acknowledges that some schools might have this instrument, but others might not; therefore in the protocol an alternative "home-made" system is provided. The system can be still used safety to run the experiment, provided a closed electrochemical cell is used (as the one described above).

In this part of the experiment students prepare films with different thickness by varying the time of synthesis (keep the same current).

In the second part of the experiment, students will study the absorbance of Prussian Blue nanoscale thin films they have produced. The absorbance of a coloured thin film at a given wavelength is expected to increase in magnitude as the film becomes thicker (Lambert Law). In this part of the experiment students will verify this is the case for the Prussian Blue thin films they have prepared.

In this experiment students will analyse the absorbance of coloured thin films. In scientific laboratories this is performed using a UV-Vis spectrophotometer adapted for thin film analysis. Such an instrument is not easily accessible to schools; therefore an alternative method is suggested, called the desktop scanner method. This method has been reported in the literature to be a valuable way of simulating the visible spectra of coloured samples (e.g. solutions) from the RGB values of their digital colour image (which give the colour intensities of red, green, and blue for pixels within the selected area in the image). This way it is possible to perform colorimetric analysis in school without a spectrophotometer. This method was selected because it allows collecting information with an acceptable level of approximation for a school level experiment, and it uses an instrument that schools already have. In this experiment students will analyse the absorbance of coloured thin films using this method, and study the relationship between absorbance and film thickness (Lambert Law).

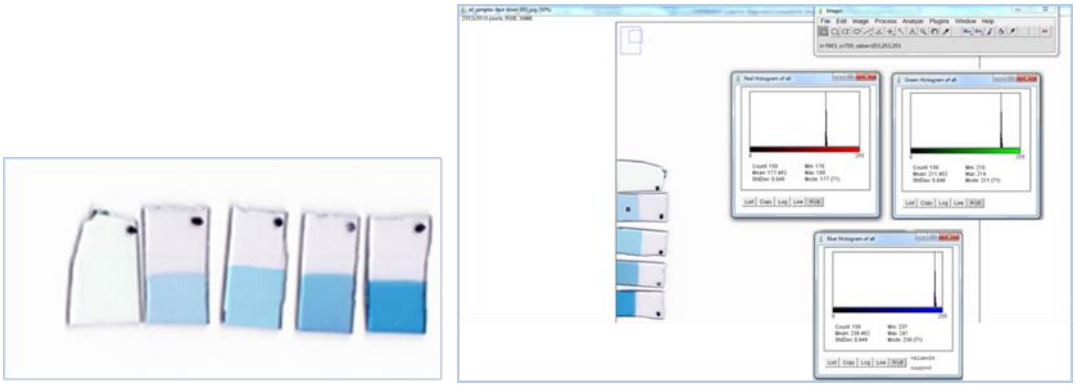

Figure 9 (Left): Four samples of Prussian Blue thin films, grown electrochemically over an ITO glass. First on the left is a piece of ITO with no film deposited. (Right): Use of the image software ImageJ to collects R, G and B values of the different thin films.
Image credit: L. Filipponi, iNANO, Aarhus University, Creative Commons Share Alike Non-Commercial 3.0.

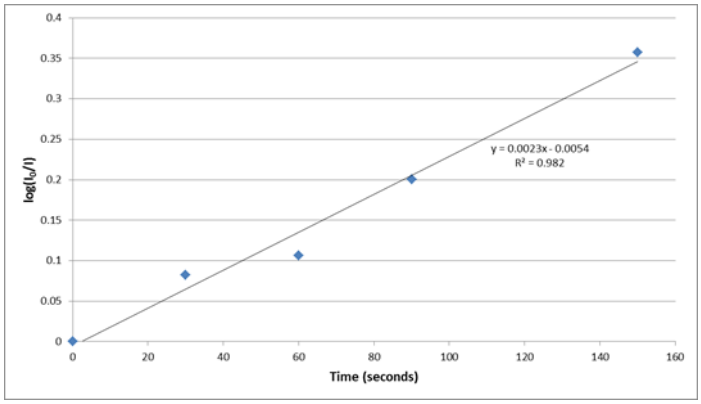

Figure 10 Absorbance plot for "red+green" light intensity by image analysis of Figure 6, using data reported in Table 2.
Image credit: L. Filipponi, iNANO, Aarhus University, Creative Commons Share Alike Non-Commercial 3.0.

In the third part of the experiment, students will use a Prussian Blue thin film prepared in the previous part of the experiment and verify this electrochromic property, specifically the electroreduction to Prussian white (WP): they will apply a positive voltage using a 1.5 V battery to the film (while this is in a KCl bath) and see the colour variation from blue to transparent. The process is reversible, and this can be seen by bringing the voltage to zero, by connecting the glass electrode directly to the graphite electrode.

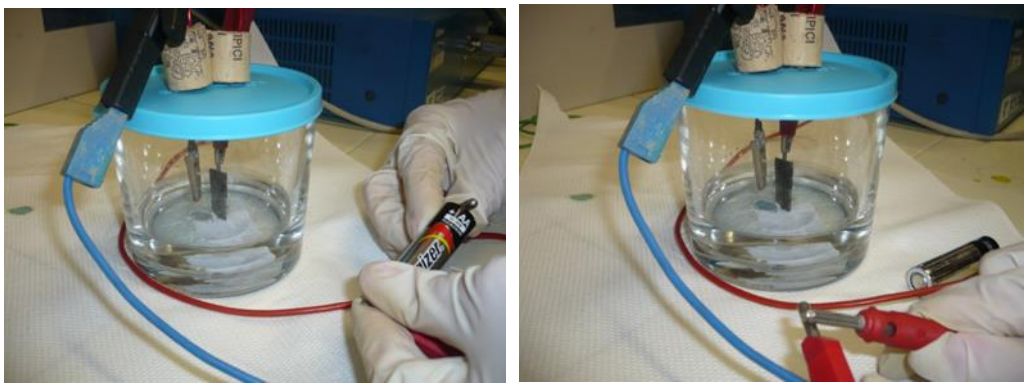

Figure 11 Testing a sample of Prussian Blue thin film for electrochromism.
(Image credit: L. Filipponi, iNANO, Aarhus University, Creative Commons Share Alike Non-Commercial 3.0).

3.2.4. Extra activity

To further complement the experiment, an extra simple activity is suggested at the end of the teacher document, called "Graphite conductivity". This activity allows teachers to show students the electrical conductivity of graphite.

Some possible **teaching goals** are:

- Illustrate students a fundamental nanoscience concept: the properties of a material depend on their nanostructure, and materials made of the same type of atoms (allotropes) can have very different properties due to the way the atoms are bond together and the nanostructures they form;

- Study some basic principles of electricity (resistance, resistivity, Ohm law).

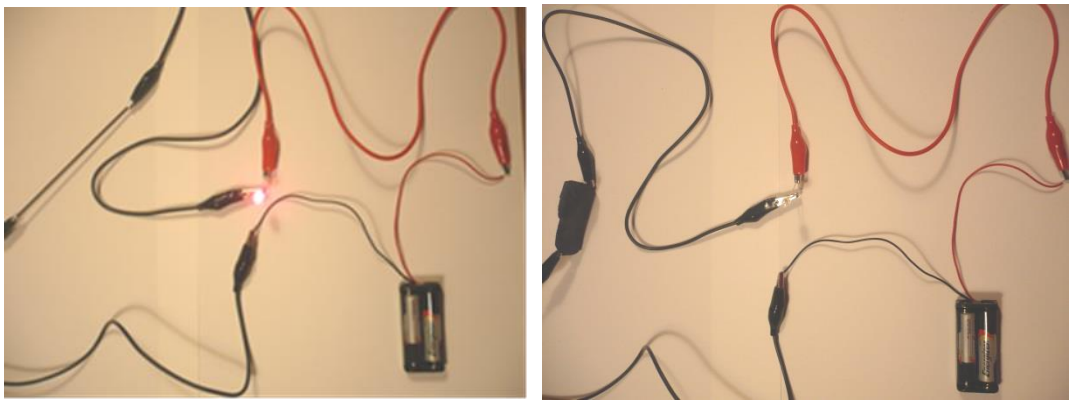

Figure 12 (Left): A graphite lead is inserted in the circuit to verify it conducts electricity. (Right): When a piece of charcoal is inserted in the circuit, the LED does not light up, confirming it does not conduct electricity.
(Image credit: L. Filipponi, iNANO, Aarhus University, Creative Commons Share Alike Non-Commercial 3.0).

3.2.5. What does the experiment teach/show about nanoscience

Thin films of nanometre thickness are studied for many reasons, one being their interesting optical properties, which can arise from different phenomena. For instance thin films of titanium oxide with nanometre thickness variations show different colours (for example, a film 100 nm is purple, and a film 30 nm is yellow). The colours are due to the interference of reflected light from the thin transparent oxide. This effect is often illustrated using soap bubbles as an example: the different colours seen are due to the different thickness of the bubbles.
In the case analysed in this experiment, the colour of the thin film is the result of the absorbance of Prussian Blue, a material which has a mixture of iron atoms in two oxidation states (FeII and FeIII) and the colour is produced by what is known as "metal-to-metal charge transfer" effect.
The electrochromic properties of Prussian Blue make this material interesting for electrochromic devices. PB thin films are used in electrochromic devices in a sandwich structure between two optically transparent conductors. When a negative potential is applied, it becomes colourless (Prussian White), and when a positive potential is applied oxidation occurs and it yields Prussian Blue. These thin films are usually combined with thin films of WO3 (another electrochromic material) in production of electrochromic devices. They are used together in a single device due to the complimentary nature of their electrochromic reactions.

Another important field of application of thin films is sensors and biosensors. Thin films in sensors and biosensors are used for many reasons, for sample handling, biological recognition and amplification, transduction and electronic signal processing. The thin can be made through a number of methods, including layer-by-layer, where specific biomolecules can even be entrapped in the film during its synthesis.

Prussian Blue thin films have been widely used in amperometric enzyme biosensors. In these type of sensors the enzyme acts by oxidising the substrate (analyte) and then returning to their original active state by transferring electrons to molecular oxygen, so that the final products are the oxidised form of the substrate (oxidised analyte) and, as a side product, hydrogen peroxide. The analyte is therefore coupled with an enzyme ("enzyme substrate").

More than ten different enzymes have been coupled with Prussian Blue in amperometric enzyme biosensors for measuring different analytes, for instance glucose oxidase (for glucose), cholesterol oxidase (for cholesterol), alcohol oxidase (for ethanol). In the food industry, amperometric enzyme biosensors coupled with Prussian Blue have been shown to be useful for monitoring food spoilage (e.g., production of ethanol) and food quality (for instance glucose is monitored in wine and beverages).

Therefore this experiment allow student to see an electrochromic substrate that is currently being used in several innovative products and applications which very possibly will reach the market in coming years (e.g., electrochromic windows).

3.3. VIRTUAL EXPERIMENT

The Virtual Experiment is being developed by IrsiCaixa in collaboration with a research group led by Dr. Pau Gorostiza from the Institute for Bioengineering of Catalonia (IBEC), and with the support of iNANO, Aarhus University. In the Virtual Experiment students will be able to get to know and work with a Scanning Tunnel Microscope. The experiment will invite students to build a molecular transistor in the same manner as Dr. Gorostiza is doing within his research line.

The experiment starts by explaining what transistors are and how they work, and discussing the visual example of an electronic circuit in a mobile phone. Here, a transistor can be used to regulate the light intensity of the lamp of the mobile camera. Then they are asked if they would like to participate in a research line to try to make transistors smaller, so that one day their mobile could be made far smaller and lighter. After this introduction students are invited to enter the lab.

First they will find information on what an STM is and what is the electron tunnelling effect, and then they are introduced to the research line to find a new generation of transistors. They are asked to choose the material they would like to use for these new transistors. Once they guess that molecules could be used, they are invited to choose the surface on which they have to place the molecules to start the experiment. Once done, then they can put it on the STM and start the experiment.

They will be able to "work" with the STM, take a first picture of the sample and settle its tip pointing at one molecule to start investigating what is the ideal distance from the molecule at which the tip should be placed. They will be able to experiment how the lamp intensity changes with time and distance to finally conclude the ideal distance to build their transistor. Then they

will operate the transistor by adjusting the "gate voltage" which allows regulating the current that passes through the lamp, just as a tap allows adjusting the water flow. In this way they will be able to change the light intensity of the lamp.

Once the molecular transistor will be build, there will be a simulation moving it into a mobile phone that will be far smaller, and this animation will come together with an explanation congratulating the student for having succeeded in the fabrication of a smaller transistor. Finally a text will appear explaining that in fact we are still far from being sure that this will happen, but that a lot of scientists all over the world are investigating in this direction.

3.3.1. What does the experiment teach/ show about nanoscience

The STM is a fundamental tool in nanoscience and nanotechnologies. It is used in both industrial and fundamental research to obtain atomic-scale images of metal and semiconducting surfaces It provides a three-dimensional profile of the surface roughness; it allows observing surface defects, and determining the size and conformation of molecules and aggregates.

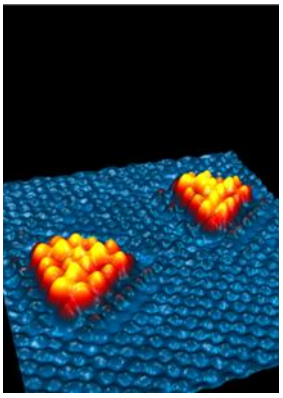

Figure 13A nanocatalyst used for cleaning up sulphur from crude oil. The STM image shows two molybdenum-disulfide nanoclusters consisting each of 15 Mo atoms and 42 S atoms. Copyright iNANO, reprint not permitted.

Another astonishing property of the STM is that it can be used to manipulate (move!) individual atoms, trigger chemical reactions, as well as performing electronic spectroscopy.

Therefore through the NANOPINION virtual experiment students have an opportunity to learn the operational principle of a fundamental instrument used in nanoscience, and try themselves to “move molecules”.
Students will work with the virtual STM with a specific application in mind, i.e., to create a nanosized molecular transistor for an innovative product, a new type of mobile phone, much smaller than current available ones. Molecular transistors are a new concept developed within the more general field of “molecular electronics”, which is based on the realization that molecules, in natural systems, (plants, and animals) are arranged in macromolecular systems (nanostructures) that perform numerous tasks which involve the transmission of charges, photons, etc. These macromolecular systems have developed though million years of evolution and are an example of excellence when it comes to efficiency of work. One example is the electric flow in nervous signalling, or the control of charges in the ionic pump, or the absorption of light and transmission of photons and charge in plant chlorophyll. The examples are numerous. Molecular electronics is a branch of nanoscience that aims to make explicit use of molecular assemblies for the transmission and storage of data. The field includes molecular wires, molecular switchers, molecular sensors and other “hardware” components of electronics. The idea is to assemble molecules in nanostructures that can perform a specific function (such as transport of charge) depending on its configuration.

APPENDIX I

KIT NAME	DESCRIPTION	COST & SHIPPING
NISENET KIT http://www.nisenet.org/nanodays-physical-kit-contents	Teachers resident in the US are eligible to receive a Physical Kit to be used primarily during a NISE NanoDay™ (pre-registration to the event is required); non-US residents, can freely download the Digital Kit (registration to nisenet.org is necessary). Both kits provide the same information about hands-on activities, and include guides and tips to help stage the NanoDays™ events. The physical kit contains all materials and supplies for each activity and includes physical signage; digital kits include downloadable guides and printable graphic files.	Free to US residents (physical kit); free to all (digital kit) participating in a NanoDays™ event.
NANOSCHOOLBOX http://www.nanobionet.de/index.php?id=139&L=2	This is a box that can be bought (entire or parts of it). The box includes simple hands-on experiments to understand basic nanoscience concepts; nanomaterials to be tested, like a memory metal and a superhydrophobic material; posters to illustrate the nanoscale dimension; and paper models of nanostructures, like a fullarene, to be built.	Online the cost declared is 279.65 Euro (to which 19% VAT must be added), plus there will be a shipping cost depending on the country. Teachers can also buy parts of the box, for instance only the ferrofluid experiment.
TIMEFORNANO KIT http://www.timefornano.eu/nanokit	The nano kit includes several hands-on activities that introduce nanotechnologies and potential applications. These activities are: • How tall are you in nanometres? How big is your hand in nanometres? • Dilution • Magnetic probe • Make your own buckyball • Ferrofluid • Magic sand • Hydrophobic textile • Anti fog • Memory metal	This kit is not for sale, only digital at the moment. The kit has been developed as part of the Time for Nano project and several activities are adapted from the NISENET kit.
GRÄTZEL CELL KIT http://www.solideas.com/solrcell/ICE_98_001_NanocrystalllineSolarCellKit.html	A Grätzel Cell is a solar cell made using nanomaterials. To build such a cell, a kit has been made by the Institute for Chemical Education. In the website teachers can also find a lot of useful info, including articles that explain in detail the Grätzel cell. The list of all materials and tools needed for this experiment can be found here: http://www.solideas.com/pdfcellkit/NanocrystallineSolarCell_Materials.pdf]	In the US it costs 45 US$, but it is more expensive in Europe Another possibility is the **experimental kit from bionik-sigma** (www.shop.bionik-sigma.de) for 16 Euro inc. VAT.

STUDENT LABORATORY WORKSHEET EXPERIMENT A: DRUG DELIVERY

Student name:……

Date:……..

AIM: The aim of this experiment is to illustrate through a simple model how a miniaturised drug delivery system is created and how the release of the drug herein contained can be controlled. The drug delivery system is created through a self-assembly encapsulation method, by using a natural polymer (alginate) and a food dye, which in the model represents the drug. In the experiment students can learn how the release of the "drug" depends from a number of variables, like the type of the drug encapsulated (this is tested by changing the food dye) and the media where the nanocapsules are released in. In addition, students test the effect of adding a "shell" to the nanocapsule to stop or slow down the release of the drug. In this experiment the "shell" is a nanocoating of chitosan, another natural polysaccharide. The coated nanoshells are also tested in different media, like water and milk (here used as a model for a complex biological media). Overall the experiment is a simple way of discussing the many variables involved in developing nanoscale drug delivery systems, and the use of nanoshells to control the delivery.

SAFETY NOTE: The chemicals used in this experiment need to be used according to MSDS specifications. Personal protection must be taken as indicated. As with all chemicals, use precautions. Solids should not be inhaled and contact with skin, eyes or clothing should be avoided. Wash hands thoroughly after handling. Dispose as indicated. All experiments must be conducted in the presence of an educator trained for science teaching. All experiments will be carried out at your own risk. Aarhus University (iNANO) and the entire NANOPINION consortium assume no liability for damage or consequential losses sustained as a result of the carrying out of the experiments described.

The research leading to these results has received funding from the European Community's Seventh Framework Programme (FP7/2007-2013) under Grant Agreement NMP.2011.3-4-290575

MATERIALS:

The items below are indicated assuming students will work in pairs. Each pair should get:

- 1 ice cube tray (preferably transparent; a normal 14 or 16 wells ice cube tray is fine)
- 5 disposable plastic pipettes (or glass pipettes)[1]
- 6 glass vials with cap with a volume of 2-5 mL (or any other small glass or plastic container holding this volume, for example a test tube)
- 50 mL calcium chloride 0.3 M (from stock solution)*
- 10 mL sodium alginate (with no food dye)*
- 10 mL sodium alginate with red food dye*
- 10 mL sodium alginate with blue food dye*
- 5 mL chitosan (from stock solution)*
- 5 mL oil*
- 50 mL distilled water*
- 50 mL full fat milk*
- 1 tweezers
- 1 empty medium size beaker (for washing)
- 1 glass rod for mixing (or a teaspoon)
- Paper towel
- Gloves
- Eye protection

*approximate volumes, does not need to be measured with precision

PROCEDURE

1. Self-assembly a drug delivery system

In natural biological processes, molecules self-assemble to create complex structures with nanoscale precision. Examples are the formation of the DNA double helix or the formation of the membrane cell

[1] A lower number of pipettes can be used if the students label them, in order to use the same pipette for the same chemical (e.g., for the alginate salt solution)

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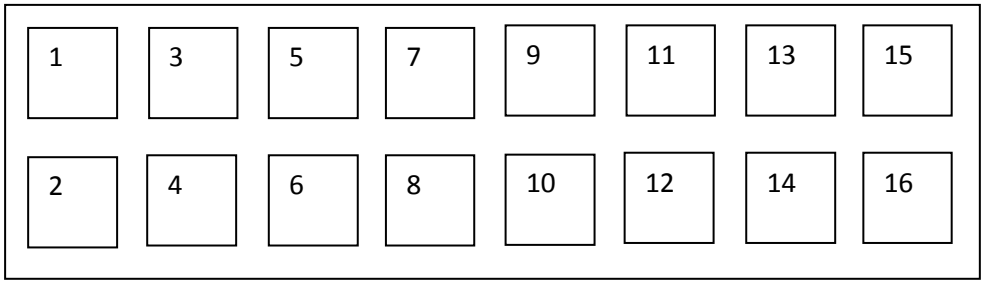
from phospholipids. In self-assembly, sub units spontaneously organise and aggregate into stable, well defined structures through **non covalent interaction**. This process is guided by information that is coded into the characteristics of the sub-units and the final structure is reached by equilibrating to the form of the lowest free energy.

In this experiment you will see how you can create some very small beads using a self-assembly process. You will be adding droplets of an aqueous solution of sodium alginate to a solution containing a high concentration of calcium ions. In this experiment your beads will mimic a "drug delivery container".

TEST 1:

Pour 5 mL of the calcium chloride solution in well #1 of your ice cube tray, and the same the same volume of water in well #2:



Using a pipette, take some of the sodium alginate solution and, from a distance of about 15 cm, add ten droplets to the calcium chloride solution (well #1). What happens?

……

Do the same over the water (well #2). Do you see any difference?

……

Q1. The alginate beads are formed because alginate self-assembles into a stable structure (a bead) in the presence of calcium ions. Why do you think this does not happen in water?

……..

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Pour 5 mL of water in well #3 and using a clean pipette, add few droplets of oil.

Q2. What happens? How can you explain the difference you see?

---

2. A model of a "drug" release system

The calcium alginate beads are a *model* of a drug delivery system. In this model, the capsule carrying the drug is represented by the calcium alginate bead, and the "drug" is represented by the food dye. The "drug" gets trapped inside the bead as it forms. Now you will test the release of the "drug" (the dye) from the calcium alginate bead in different media, like water and milk.

What do you think could happen?.....

TEST 2:

Make some red calcium-alginate beads: Pour 5 mL of the calcium chloride solution in well #4 of your ice cube tray. Using a clean pipette, take some of the sodium alginate solution (which contains the **red dye**) and, from a distance of about 15 cm, add ten droplets to the calcium chloride solution. What happens?

With a tweezers, take 5 of the red beads you have just made and gently place them in a glass vial or a glass test tube that contains 2 mL of distilled water. Take the other 5 red beads and place them in a glass vial or glass test tube containing 2 mL of full fat milk. Close vials or cover the test tube with some wrapping plastics. Rinse the tweezers with water.

Write down your start time:……

Record your observations in the table below.

	After 15 min	**After 30 min**	**After 1 hour**	**Next day***
Red Beads in water				
Red Beads in milk				

*you need to compare at the same time the following day, e.g., after 12 hours

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Q3. Has the red dye inside the beads been released in water? How long did it take?

…….

Q4. Has the red dye inside the beads been released in milk? How long did it take? Is there a difference compared to what you observe in water?

…….

Q5. If you see a difference, write down here your hypothesis for explaining it (think about water and milk, what similarities and differences they have, etc.)

……

……..

Q6. Do you think the interaction between the red dye and the calcium alginate polymer influences the release of the dye from it? What could it happen if you changed the dye?

……

TEST 3

Now make some blue calcium-alginate beads: repeat the procedure you followed in TEST 2, but this time use the sodium alginate solution which contains the **blue dye**.

Do you think changing the colour of the food dye can influence the way it diffuses out of the calcium alginate bead?.....

Pour 5 mL of the calcium chloride solution in well #5 of your ice cube tray. Using a clean pipette, take some of the sodium alginate solution (which contains the **blue dye**) and, from a distance of about 15 cm, add ten droplets to the calcium chloride solution.

With a tweezers, take 5 of the blue beads you have just made and gently place them in a glass vial or a glass test tube that contains 2 mL of distilled water. Take the other 5 blue beads and place them in a glass vial or glass test tube containing 2 mL of full fat milk. Close vials or cover the test tube with some wrapping plastics.

Record your observations in the table below.

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Write down your start time:……

	After 15 min	After 30 min	After 1 hour	Next day*
Blue Beads in water				
Blue Beads in milk				

*you need to compare at the same time the following day, e.g., after 12 hours

Q7. Has the blue dye inside the beads been released in water? How long did it take?

…….

Q8. Has the blue dye inside the beads been released in milk? How long did it take? Is there a difference compared to what you observe in water?

…….

Q9. Is there a marked difference between what you observed using the red-alginate beads, and the blue alginate beads?

……

Q10. Write here your hypothesis to explain the different behaviour you have observed between red and blue beads:

……..

Q11. What could you do to slow down, or stop, the release of the blue dye from the alginate bead?

……

3. Controlled release

To control the release of the encapsulated drug (in this model represented by the "dye"), one possibility is to coat the capsule carrying the drug with an outer layer. In this experiment, you will create a

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nanocoating of chitosan, a natural polymer, and test if and how it affects the release of the dye from the calcium alginate bead.

What do you think the effect of the chitosan nanocoating will be?.....
.....

For this part of the experiment **it is suggested to throw away all liquid contained in your ice cube tray and start with a clean one**. Hence, throw liquids in a sink, rinse with water, dry with some paper and start with a clean ice cube tray.

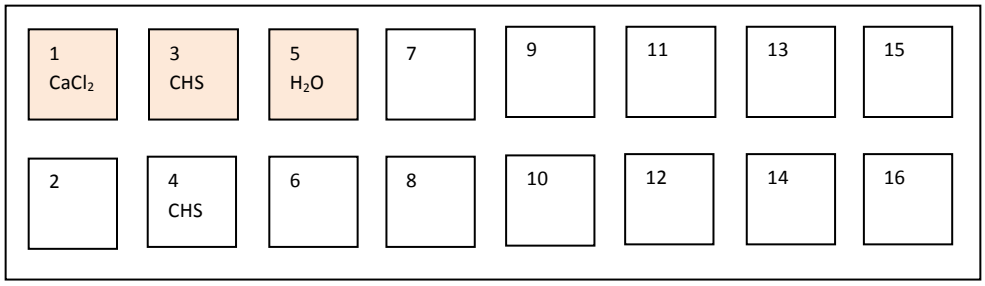
TEST 4:

Prepare two vials: put 2mL of water in one glass vial (or test tube), and 2mL of milk in the other glass vial (or test tube). Set aside but remember to write on them “chitosan- water” and “chitosan-milk” with a permanent marker, respectively.

Take 2mL of chitosan, add it in well #3 of you ice cube tray and add to it 2 mL of distilled water. Mix with a glass rod or with a teaspoon. Do the same in well #4 of your ice cube tray: first add 2mL of chitosan, than 2mL of water to it, and gently mix. Now you have two solutions of chitosan ready to be used.

Make 5 new blue calcium alginate beads following the procedure you have done before.

Pour 5 mL of the calcium chloride solution in well #1 of your ice cube tray and 5mL of distilled water in well #5. This is how your solutions should be distributed. In the scheme “CHS” stands for “chitosan solution”. Highlighted in orange are the wells you will be using now:



Using a clean pipette, take some of the sodium alginate solution (which contains the **blue dye**) and, from a distance of about 15 cm, add five droplets to the calcium chloride solution in well #1.

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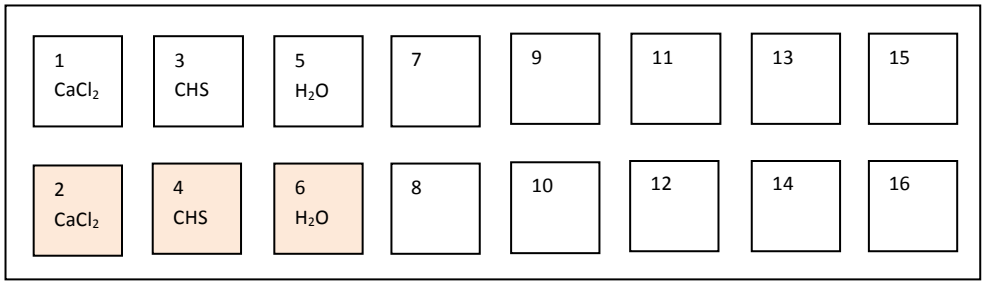
Now quickly take 5 blue beads and place them in well #3 (containing chitosan). Leave the beads to incubate for 5 min. Have the glass vials with written “chitosan-water” ready to be used and a piece of paper towel next to the tray.

After 5 minutes, take quickly out the beads from well#3 (chitosan solution) : take one, dip it quickly in well #5 containing water (only to rinse off the excess of chitosan), touch the paper towel (to remove the excess of liquid), and place the bead in the vial that says “chitosan-water”. **Record start time here**:……

Now do the same for the other 5 new beads.

Pour 5 mL of the calcium chloride solution in well #2 of your ice cube tray and 5mL of distilled water in well #6. This is how your solutions should be distributed. In the scheme “CHS” stands for “chitosan solution”. Highlighted in orange are the wells you will be using now:



Using a clean pipette, take some of the sodium alginate solution (which contains the **blue dye**) and, from a distance of about 15 cm, add five droplets to the calcium chloride solution in well #2.

Now quickly take 5 blue beads and place them in well #4 (containing chitosan solution). Leave the beads to incubate for 5 min. Have the glass vials with written “chitosan-milk” ready to be used and a piece of paper towel next to the tray.

After 5 minutes, take quickly out the beads from well#4 (chitosan solution) : take one, dip it quickly in well #6 containing water (only to rinse off the excess of chitosan), touch the paper towel (to remove the excess of liquid), and place the bead in the vial that says “chitosan-milk”. **Record start time here**:……

The research leading to these results has received funding from the European Community's Seventh Framework Programme (FP7/2007-2013) under Grant Agreement NMP.2011.3-4-290575

Record your observations in the table below.

	After 15 min	After 30 min	After 1 hour	Next day*
Blue Beads Chitosan Coated- in water				
Blue Beads Chitosan Coated- in milk				

Q12. What was the effect of coating the beads with chitosan?

……

Q13. Did the coating stop the release of the dye in water and milk in the same way? How can you explain this difference?

……

……

EXTRA TEST (Optional)

Q14. If calcium alginate beads need a solution of calcium to be formed, do you think you could use milk instead of the solution of $CaCl_2$? Do you think it could work? Write your answer and your hypothesis:

……..

……..

Now add 5 mL of milk to well # 8 and try it! Add few droplets of the alginate solution (containing the red dye) to the milk, and see what happens. Did beads form?

……

You can discuss with your teacher your initial hypothesis and your observations.

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NANOPINION Educational Resources

Experiment A – Controlled Drug Delivery

14-18 years

Written by Luisa Filipponi (iNANO)
Interdisciplinary Nanoscience Center
Aarhus University, Denmark
August 2013

MATERIAL INCLUDED IN THIS EXPERIMENT PACKAGE:

For teacher:

TEACHER RESOURCES FOR EXPERIMENT A

For students:

STUDENT LABORATORY WORKSHEET EXPERIMENT A

LEVEL OF EXPERIMENT: Simple

DISCLAIMER: The experiments described in the following training kit use chemicals which need to be used according to MSDS specifications and according to specific school safety rules. Personal protection must be taken as indicated. As with all chemicals, use precautions. Solids should not be inhaled and contact with skin, eyes or clothing should be avoided. Wash hands thoroughly after handling. Dispose as indicated. All experiments must be conducted in the presence of an educator trained for science teaching. All experiments will be carried out at your own risk. Aarhus University (iNANO) and the entire NANOPINION consortium assume no liability for damage or consequential losses sustained as a result of the carrying out of the experiments described.

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EXPERIMENT A: CONTROLLED DRUG DELIVERY

SUMMARY: The aim of this experiment is to illustrate through a simple model how a miniaturised drug delivery system is created and how the release of the drug herein contained can be controlled. The drug delivery system is created through a self-assembly encapsulation method, by using a natural polymer (alginate) and a food dye, which in the model represents the drug. In the experiment students can learn how the release of the "drug" depends from a number of variables, like the type of the drug encapsulated (this is tested by changing the food dye) and the media where the nanocapsules are released in. In addition, students test the effect of adding a "shell" to the nanocapsule to stop or slow down the release of the drug. In this experiment the "shell" is a nanocoating of chitosan, another natural polysaccharide. The coated nanoshells are also tested in different media, like water and milk (here used as a model for a complex biological media). Overall the experiment is a simple way of discussing the many variables involved in developing nanoscale drug delivery systems, and the use of nanoshells to control the delivery.

FIELD OF NANOTECHNOLOGY APPLICATION: Nanomedicine

TEACHING GOALS:

- Familiarize students with the concept of self-assembly as a method to create nanomaterials, in particular nanocapsules, and the forces that are involved in this process
- Discuss the variables that scientists need to consider when designing a drug delivery system
- Study how some variables, like drug composition (chemical structure, solubility, charge, etc.), nature of the capsule and its outer shell, and media, influence the diffusion of the "drug" from the drug delivery system
- Discuss the effect of the "shell" and how a core-shell system can be used to control drug delivery, either passively or actively

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BACKGROUND INFORMATION

Controlled drug delivery

Conventional pharmaceutical drugs are limited by problems such as low efficacy, low solubility in water and lack of selectivity. In addition, physiological barriers often prevent the drug from reaching and acting at the target site – a phenomenon called drug resistance. The low solubility and limited bioavailability of conventional drugs is responsible for their limited effectiveness: the body often clears away the drug before its action has fully occurred. The efficacy of drugs is also dependent on the dose used, but dose-dependent side effects often limit their acceptable usage. The lack of selectivity is especially detrimental for instance in cancer therapies, since anti-cancer drugs, usually used in large volume of distribution, are toxic to both normal and cancer cells.

A recognised need exists to improve drug composition, delivery, release and action, and thus to develop new drugs that can act at the specific site of the disease, maximising the drug's therapeutic action while minimising side effects. For drugs to be able to do so, the delivery systems need to be miniaturised to become much smaller than the target, and specific in composition to elicit a certain response. With the use of nanotechnologies, targeted drugs (in terms of composition and delivery system) are becoming a reality: these are normally called nano-drug delivery systems. In the future this could lead to targeted therapies and personalised medicine. The aim is to design nano-drug delivery systems with optimal loading and deliver them in such a way that they can recognise the "bad" cells at a molecular level, penetrate the cell membrane and act inside the infected cell. This is often crucial for the efficacy of a drug, since most virus replication and other disease conditions take place across the cell membrane and inside the cell. This way, the treatment will be delivered where is needed, in the right amount and will be specific, eliminating the problem of the drug killing healthy cells and reducing side effects for the patients.

Drug safety can be further enhanced by the possibility of introducing inside the drug formulation a label that changes colour when the drug reaches its expiry date or is no longer functioning. This will allow the improvement of drug shelf-life and better monitoring of drug safety.

Nano-sized drug carriers that are currently under development include either materials that self-assemble, or conjugated multicomponent systems, for instance a drug linked to a protein and a polymer (called polymer-drug conjugate). Numerous nanosystems are now being investigated, and include micelles, nanoemulsions, nanotubes, nanofibres, liposomes, dendrimers, polymer therapeutics, nanoparticles, nanocapsules, nanospheres and hydrogels. Some of these nano-sized drug carriers are established in the field of drug delivery, such as liposomes; others have made their way to the market in recent years, such as polymer-protein conjugates (polymer pharmaceutics). Many are now used for treating some forms of cancer, hepatitis, and leukaemia. An example is an anti-cancer drug called DOXIL (Sequus Pharmaceuticals) or Emend®, an anti-nausea drug for chemotherapy patients (Merk & co.) In

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the case of DOXIL the drug (doxorubicine) is encapsulated inside a liposome, which is then protected by a layer of polyethylene glycol.

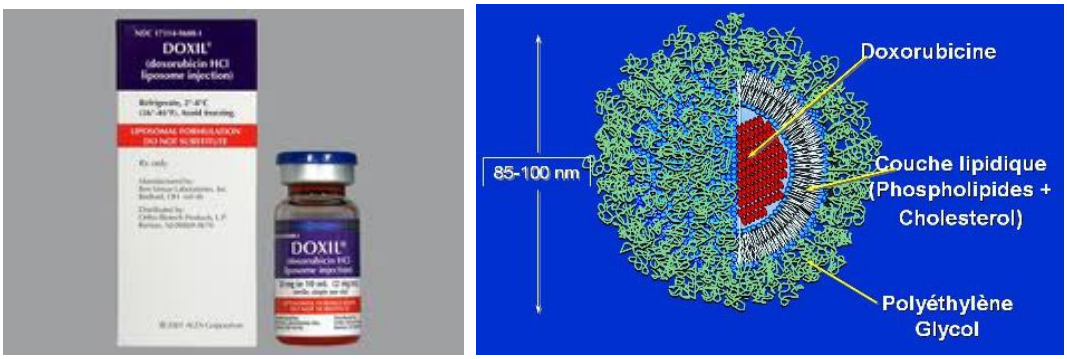

Figure 1. (Left) The commercial drug DOXIL®; (right): encapsulation technology used in the drug DOXIL®

Encapsulation and release

The general concept behind many novel drug delivery systems is to "trap" the drug inside a capsule, which acts as a carrier to deliver the drug at the target, in some cases passing through the cell barrier and reaching the inside of the infected cell. The capsule has a protective shell that prevents the capsule from being dissolved before reaching the target. In addition, some biomolecules can be attached to the outer shell, which are specific to the target.

Figure 2 provides a schematic overview of encapsulation technology used for drug delivery.

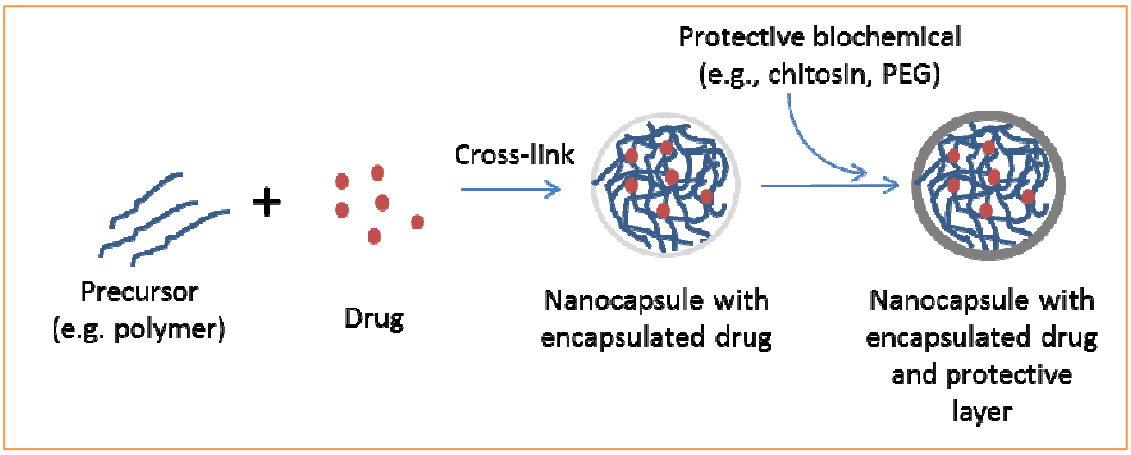

Figure 2. Overview of encapsulation technology. L. Filipponi, Creative Commons Non-Commercial Share Alike 3.0

In the example illustrated in Figure 2 the nanocapsule is formed by self-assembly, using a material, for instance a polymer that cross-links in certain conditions and entraps the drug during the self-assembly

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process. The protective layer (often called a "shell") can act as an anti-fouling agent; can have specific target molecules that identify and reach the target; can have specific solubility properties, i.e., be degraded by enzymes that are located only at the target site or be stable only in specific acidic conditions.

It is clear that from a material engineering point of view, the development of such drug delivery systems requires a detailed understanding of the chemical and biochemical properties of the capsule and the environment it will be moving into and dissolving at (e.g., pH, presence of specific enzymes, etc.), as well as a detailed understanding of the physical process (diffusion rate and diffusion motion, etc.)- a truly interdisciplinary work!

In an even more advanced level, scientist are even looking at ways to include inside the capsule not the drug, but the DNA and other biological machinery for making the drug! [see Reference 4]

The future of targeted drug delivery enabled by nanotechnologies could be miniaturised implantable chips loaded with different drugs that can be released upon external stimuli, or upon reaching the target, and the active component released at a controlled rate. This is called stimuli-activated drug delivery. Controlled activation could be linked to some environmental properties, such as pH, or "lock-and-key" molecular recognition mechanisms. This could free patients such as diabetics from having to administer drugs repeatedly during the day.

Integrating treatment and diagnostics: Theranostics

Nanotechnology could bring drug delivery even a step further by integrating the diagnosis, therapy and follow-up of a disease. This is referred to as "theranostics", and could be enabled by nanoparticles incorporated inside a drug that can change some property –such as colour – once the drug has reached the target (for instance, quantum dots). Drugs could therefore have a feedback action. Together with a slow, targeted release system, the nanoparticles could gradually change colour during the drug action, thereby informing doctors of the progress of a therapy. This approach is called "find, fight and follow" and could become a reality thanks to the parallel progress of the field of molecular imaging. One vision is that, one day, the entire process of diagnosis, pre-imaging, therapy and post-imaging of a specific disease will be integrated. An example of theranostics is the use of gold nanoshells for imaging and treating cancer cells at the same time. In this case the "shell" has a double function: it is a targeting and an imaging tool.

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OVERVIEW OF THE EXPERIMENT

1. Capsules self-assembly

In the first part of the experiment students will learn a simple way of making "capsules" that in the second part of the experiment will be used as "drug carriers". The capsules form through a cross-linking process that occurs spontaneously when mixing a solution of alginate salt and calcium chloride. A regular, spherical structure is formed (in this document called "bead"), hence this is a simple example of self-assembly. The capsules formed are not nanosized but rather micron-sized. However, the same material is used by scientists to create nanocapsules.

Why do capsules from? Sodium alginate is a polymer obtained from seaweed. It has a linear structure with many carboxyl groups sticking out (a "carboxyl group" is a combination of carbon atoms and two oxygen atoms carrying a *single negative charge*). When a solution of sodium alginate is combined with a solution of calcium chloride, the calcium ions (Ca^{2+}, with a *double positive charge*) are able "bridge" two different alginate strands. The result is a cross-linked polymer which has a gel-like consistency (**Figure 3**).

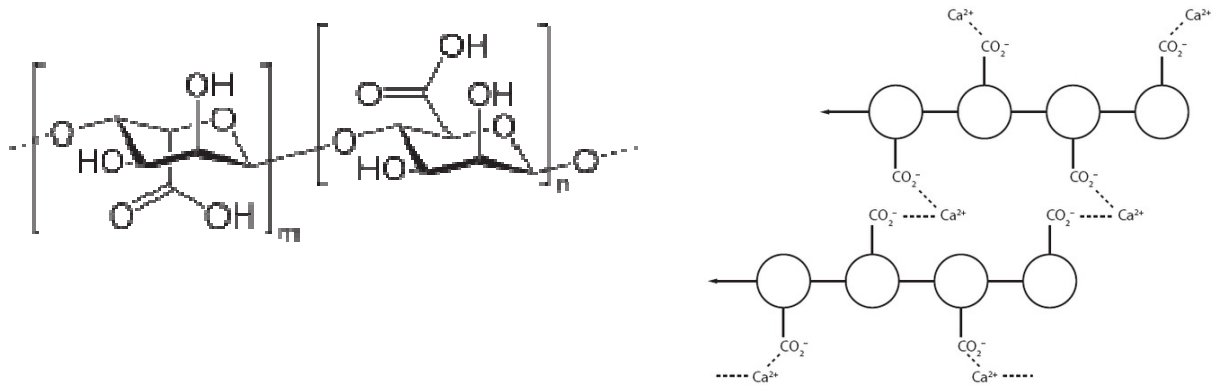

Figure 3. (Left) Sodium alginate structure (repeat unit); (right) schematic representation of sodium alginate cross-linked polymer (through calcium atoms).

Beads form immediately in $CaCl_2$; the shape of the bead is spherical unless the droplet is released too closely/low above the $CaCl_2$ solution (in which case it can't adopt fully spherical shape before it hits the surface of $CaCl_2$ solution and forms a "tail").

To appreciate this process, students can try making the beads in solutions alternative to $CaCl_2$ that they probably expect to contain some Ca^{2+} ions, for instance tap water and milk. In both cases, beads don't form. Milk is suggested because students will have the general knowledge that milk "contains calcium". However, milk is not a solution of calcium ions, rather a *colloid*, where calcium ions are trapped inside

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nanostructures and are not freely available (this aspect can be used to discuss in class the difference between an ionic solution and a colloid)[1].

2. Drug encapsulation and release

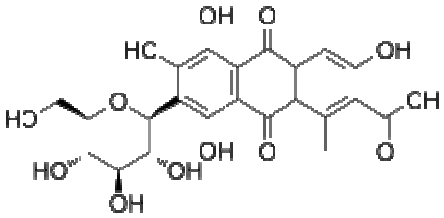
In the second part of the experiment, students will encapsulate a "drug" inside the calcium-alginate capsules. This process is done during the self-assembly step, since the "drug" (in this model represented by a *food dye*) is added to the alginate salt solution. The alginate mixture (having a strong colour) in now added drop-wise to the $CaCl_2$ solution, as described previously. Coloured beads are formed.

The dye is trapped inside the calcium alginate beads, and its diffusion in water and milk is compared. Students should make some hypothesis on the fact that this process depends on the type of media the beads are placed in and on the type of the dye used. In this experiment the milk is used to simulate a biological fluid (contains proteins, minerals, fat molecules), therefore a much more complex media than water.

Two systems are compared, one using a red dye and one using a blue dye. The two food dyes differ for chemical structure and charge. The blue dye diffuses rapidly from the bead (after 15 minutes release is observed) whereas the red dye is trapped strongly.

Understanding the different behaviour of red food dye and blue food dye

The different diffusion behaviour of the red and blue dye is most likely due to the difference in chemical structure and charge between the two materials. **Figure 4** provides the chemical structure of the dyes contained in the food dyes solutions:



Food dye: E120 (carminic acid)

[1] The project NANOYOU has developed an experiment where milk, and other natural colloids, are studied in details. A colloid is an example of a nanomaterial.

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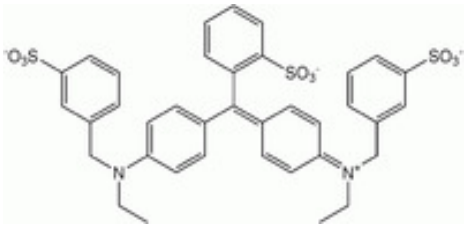

Food dye: E133 (Brilliant Blue)

Figure 4. The chemical structures of red and blue food dye used in this experiment

As it can be seen, the two dyes have a very different chemical structure. It is not the intention of the author to investigate in details this matter, which requires an advanced understanding of chemistry, not suitable for the age group at which this experiment is targeted. The suggestion to teachers is to raise the issue of chemical difference between the dyes focusing on how this might influence the way the dye is "trapped" inside the bead and its "willingness" to detach from it. The ultimate aim is to get students to realize that, when designing a drug delivery system, scientists need to consider numerous variables, among which the type of drug that needs to be encapsulated and carried: the capsule should carry the drug but then eventually release it!

3. Adding a shell & controlling the release

In the third part of the experiments, students add a "shell" to the beads and study how this affects the release of the food dye. The shell is made of chitosan, a natural polysaccharide which come from the shell of crabs and other crustaceous.

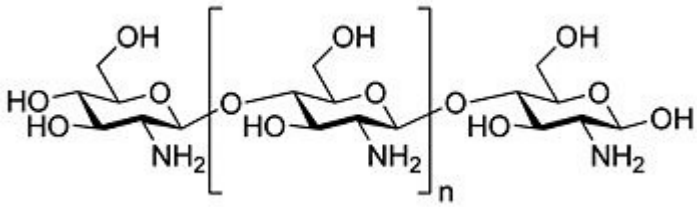

Figure 5. Chemical structure of chitosan

Students will observe that adding an outer nanocaoting to the calcium alginate bead strongly influences the dye release. This effect is seen when using both the red and the blue dye, but it is most notable in the latter case.

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Understanding the effect of the chitosan shell

The amino group in chitosan has a pKa value of ~6.5, which leads to a protonation in acidic to neutral solution; for this reason chitosan needs to be solubilised in an acidic solution (in the experiment you will use citric acid). This makes chitosan water soluble and a bioadhesive: it readily binds to negatively charged surfaces. Therefore a chitosan shell will maintain its structure in a neutral environment, but will solubilize and degrade in an acidic environment. This property is used in controlled drug delivery: a drug capsule with a chitosan shell can be used to transport a drug (for instance, insulin) to an acidic environment, where the chitosan coating will then degrade, releasing the drug to the desired environment. It should be noted that the release depends also on other factors like molecular weight of the drug, chemical composition and conformation, charge etc.

In this experiment you will test the effect of adding a nanocoating of chitosan to beads containing the blue dye. The shell prevents the blue dye from diffusing out of the alginate beads when there are soaked in water (neutral environment). It is suggested to use the blue dye alginate beads because the dye diffuses out from the bead very quickly (in 30 minutes) when no shell is added to the bead, so even after a relatively short time, students can see the effect of the chitosan coating.
The effect of the shell is different when the beads are placed in milk (which is slightly acidic). Milk also contains enzymes and numerous biomolecules that most likely degrade the chitosan shell. The process is slow, so it is suggested that it is observed after several hours (e.g. after overnight).

Comparing the effect of the chitosan shell in water and milk is therefore an effective way to show students the importance of choosing the right coating for a drug delivery system, with respect to the environment it need to travel to, and the location where the drug needs to be released (other factor that the teacher can mention is time: in some cases the drug needs to be delivered quickly once it reaches the target site; other times slowly. This requires a lot of material engineering!)

THIS EXPERIMENT IN CLASS

The present experiment is a *model* of a drug delivery system. By “model” it is meant:

- The nanocapsule is represented by a macroscopic sphere (referred as “bead”) which is made of a naturally occurring polymer, alginate
- Like for many nanocapsules developed in research, the alginate bead is formed by self-assembly
- The drug is simulated by a dye, which gets entrapped in the bead during the self-assembly process
- The release of the drug (i.e., the dye) is studied in two media, water and milk. Milk simulates a biological fluid, since milk contains many different biomolecules and enzymes
- The control of the release is simulated by placing a nanocoating on the outer surface of the bead. The nanocoating is made of chitosan, another natural occurring polymer. The resulting

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system simulates a "core-shell" system, where the "shell" acts as a protective layer, and the "core" carries the drug

To make the most of this experiment, it is recommended using it together with the **nanOpinion Moodle minicourse called "Drug Delivery & Theranostics"**, where students can see some practical applications of controlled drug release using a core-shell nanoparticle (http://nanopinion-edu.eu/).

MATERIALS AND STOCK SOLUTION

Chemicals (can be all bought from Sigma-Aldrich, www.sigmaaldrich.com/):

NAME	QUANTITY	SIGMA PRODUCT NUMBER/ CAS NUMBER/ APPROX. COST	QUANTITY NEEDED FOR 20 STUDENTS*
Calcium chloride dihydrate	500 gr	223506 [10035-04-8] 22 Euro	22.05 gr
Alginic acid sodium salt	100 gr	180947 [9005-38-3] 38 Euro	6 gr
Chitosan Low MW	50 gr	448869 [9012-76-4] 79 Euro	1 gr
Citric Acid	100 gr	251275 [77-92-9] 29 Euro	2 gr

*Assuming 20 students working in couples, so 10 groups

Other materials needed, can be found at any supermarket:

Full Fat Milk	1 L
Distilled water	2 L
Tap water	1 L
Oil	1 cup
Red Food Dye (E120, Carminic acid), e.g. Dr Oetker Food Colouring Red	1 bottle
Blue Food Dye (E133, Brilliant Blue), e.g. Dr Oetker Food Colouring Red	1 bottle

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Before starting the experiment with the class, the teacher should prepare the following stock solutions:
- **calcium chloride solution** (0.3 M): 22.05 gr in 500 mL in distilled water
- **sodium alginate solution**: dissolve 6 gr in 300 mL hot tap water. NOTE: add the alginate slowly as some lumps could form, stir the solution while preparing it. If lumps form, don't worry, but don't use them for making the beads. Roughly divide the amount in 3 beakers (approx. 100 mL each) and add food dye to two (4 droplets).
- **chitosan solution**: 1gr added to a solution of 50 mL of distilled water having 2 gr of citric acid mixed in

LAB EQUIPMENT NEEDED

For the entire class:

Scale with 0.1 g precision

CONSUMABLES AND MATERIALS FOR STUDENTS

The items below are indicated assuming students will work in pairs. Each pair should get:

- 1 ice cube tray (preferably transparent; a normal 14 or 16 wells ice cube tray is fine)
- 5 disposable plastic pipettes (or glass pipettes)[2]
- 6 glass vials with cap with a volume of 2-5 mL (or any other small glass or plastic container holding this volume, for example a test tube)
- 50 mL calcium chloride 0.3 M (from stock solution)*
- 10 mL sodium alginate (with no food dye)*
- 10 mL sodium alginate with red food dye*
- 10 mL sodium alginate with blue food dye*
- 5 mL chitosan (from stock solution)*
- 5 mL oil*
- 50 mL distilled water*
- 50 mL full fat milk*
- 1 tweezers
- 1 empty medium size beaker (for washing)
- 1 glass rod for mixing (or a teaspoon)
- Paper towel
- Gloves
- Eye protection

*approximate volumes, does not need to be measured with precision

[2] A lower number of pipettes can be used if the students label them, in order to use the same pipette for the same chemical (e.g., for the alginate salt solution)

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SAFETY NOTE: This experiment doesn't use chemicals but only common liquids and solids. Nevertheless staining is possible so wash hands and surfaces thoroughly after handling. Use appropriate clothing protection, gloves and eyes protection. Collect all liquids and washing water is glass/plastic containers and dispose of in sink. All experiments will be carried out at your own risk. Aarhus University (iNANO) and the entire NANOPINION consortium assume no liability for damage or consequential losses sustained as a result of the carrying out of the experiments described.

PROCEDURE

Before starting the experiment in class, teachers should prepare the **stock solutions** (see above).

NOTE! Teachers can use the Students Worksheet while doing this experiment. The Worksheet has some questions along the protocol to encourage an enquiry-based approach. However, teachers can adjust the questions and the tables in the Student Worksheet to suit their needs and/or to add questions you would like the students to answer (depending on teaching approach, subject, age, etc.).

Did you know? *There is a new trend in modern cooking that is called "food spherification". Chefs are using solutions of alginate salt mixed with some juices, or even chocolate, to make "spheres" of food. This can be an interesting example to show in class of how sometimes science is used out of the lab...and in cooking!*

1. Self-assembly a drug delivery system

TEST 1: In the first part of the experiment students will learn a simple way of making "capsules" that in the second part of the experiment are used as "drug carriers". The capsules form through a cross-linking process that occurs spontaneously when mixing alginate salt and calcium chloride. A regular, spherical structure is formed (in this document called "bead"), hence this is a simple example of self-assembly

NOTE: To create the calcium-alginate beads you will need a container of plastic that can hold about 5mL. It is suggested to use an ice cube tray as this is formed of different "wells" that can be used as reactors. The Student Worksheet has a scheme that should help students remembering what solution to put in the different wells. You can change this depending on the ice cube tray (or other container) you are using.

A solution of alginate salt is added to a solution of $CaCl_2$ using a pipette, drop-by-drop, from a distance of about 15cm. Students will need only few droplets, so you can give a small beaker with some solution (with no food dye). As the drop falls into the $CaCl_2$ solution, an opaque-white bead is form immediately.

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The beads form by self-assembly: the calcium ions are "trapped" inside the alginate salt and form a stable structure.

Students are asked to repeat the test using tap water instead of $CaCl_2$ and as an additional (optional) activity they can try to make them in milk (see page 8 of Student Worksheet).

NB. Since milk is white and opaque, it is suggested to try this test using the red alginate solution. Students will observe that beads do not form, and this can lead to a discussion on why this happens (milk is not a calcium solution, etc.)

Figure 6. Calcium beads do not form in milk.

Finally, it is suggested to compare the formation of calcium-alginate spheres with what happens when oil droplets are dropped in water. In this case, the oil self-assembles in round-shaped flat films, as a result of hydrophobicity. The oil molecules (hydrophobic) try to minimize the interaction with water molecules (hydrophilic). This is the same process seen in micelle formation. Although it is a self-assembly process, it differs from the formation of calcium alginate beads. In the latter case, the calcium ions in water interact with the alginate polymer, cross-link and form stable structures. In the case of oil, its molecules try to minimize the interaction with water, and in doing so "assemble" in a structure where this interaction is minimized.

Students should complete questions Q1 and Q2 in the Student Worksheet.

2. A model of a "drug" release system

In this second part of the experiment, students will create a model of a drug delivery system, where the capsule is made of calcium-alginate and the "drug" is simulated by a food dye. In this part of the experiment you should give each couple of students a small beaker with some red alginate solution and another beaker with some blue alginate solution (it is not important how much you give them, they will need only few droplets, 5 mL in each beaker is more than enough).

As a first thing, students will prepare some **red calcium-alginate beads**.
Students then compare the effect of immersing the beads in water and in milk, for different times. It is suggested to encourage students to think about the possible effects this could have before running the test, what variables could be important (time, temperature, pH etc.). After writing their hypothesis, students should run the test (**TEST 2**), and write down their observations.

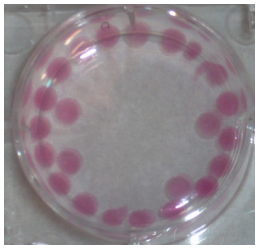

Figure 7. Calcium alginate beads with a red food dye entrapped.

Students will observe that the red dye is stable inside the bead, no release is observed after relatively short times. However if the beads

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are left in water overnight, some release is observed.

To (visually) estimate the amount of release, it is suggested to remove the surnatant from the vial, place it in a new vial and compare it with a control (containing only water), as shown in Figure 8.

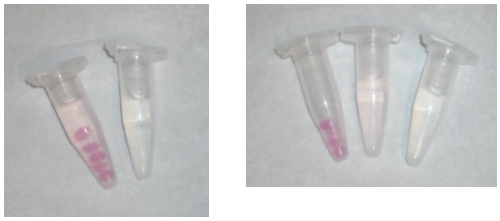

Figure 8. Red calcium alginate beads in water after overnight immersion (left); comparison between beads after overnight immersion in water, surnatant, and control (right vial, contains only water)

The beads left in milk have also release some dye, and comparing the beads extracted from water with those extracted from milk shows a clear difference. After being soaked in milk overnight (Figure 9):

Figure 9. (Left): Red calcium alginate beads in milk after overnight immersion; (Middle): comparison between beads after overnight immersion in milk, surnatant, and control (right vial, contains only milk); (Right): Red calcium beads after overnight immersion in water (left vial) and in milk (right vial); Inset shows the a close view of these same beads (left bead: overnight immersion in water; right bead: overnight immersion in milk)

Students should complete questions Q3 to Q6 in the Student Worksheet.

The importance of the type of "drug" entrapped in the capsule is investigated in **TEST 3**, where students compare what happens when a different food dye is used (blue food dye). As with **TEST 2**, it is encouraged that students think what the effect of using a food dye with a different "colour" might be. Depending on the age and level of the class, teacher can discuss how "colours" in many materials

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depend on the presence of pigments, i.e., organic molecules that absorb visible light[3]. Then they prepare some **blue calcium-alginate beads** and then repeat the same diffusion test in water and in milk.

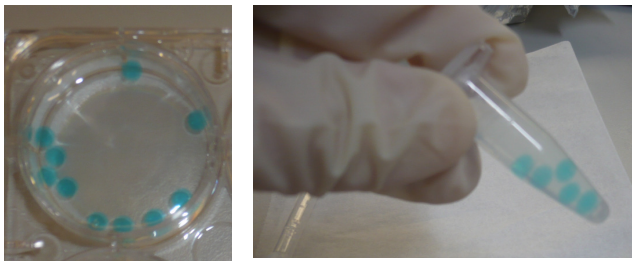

Figure 10. Calcium alginate beads with a blue food dye entrapped.

It should be noted that the blue dye diffuses very quickly out of the calcium alginate beads, after 15 minutes a clear difference is noted compared with the red calcium alginate beads. Students are encouraged to reflect upon why this happens and should come to the conclusion that the chemical structure of the dye and its interaction (or lack of) with the calcium alginate polymer must be different (variables are charge, pKa of functional groups, conformation, hydrogen bridges...).

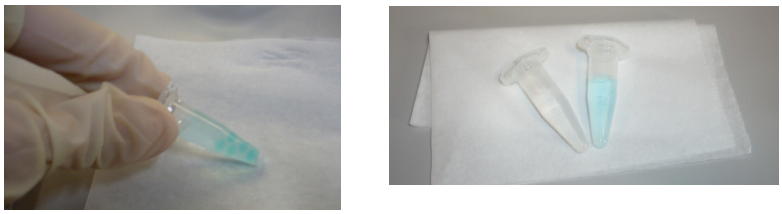

Figure 11. Blue calcium alginate beads in water: after 30 minutes of being left in water (left) and after 2 hours (right). In the right image the vial on the left contains only water as control.

Students should complete questions Q7 to Q11 in the Student Worksheet.

3. Controlled release

In the third part of the experiment, a chitosan shell is added to the beads, and the effect on dye release from the bead is observed, both in water and in milk. **Hence the results of TEST 3 should be considered the "control" for this part of the experiment**[4].

[3] This can lead to an interesting discussion on what confers "colour" to a material, i.e. the difference between colour due to the absorption by organic molecules (e.g., a dye) and colour arising by the interaction of light with nanoscale features in a material (scattering, interference).

[4] Hence it is suggested to take pictures to compare results! Alternatively, you can have the students create a new control for this part of the experiment (i.e., they should make some new blue calcium-alginate beads and place them in a vial containing water and another set in a vial containing milk).

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In order to avoid confusion with the different liquids, it is suggested that students use a clean ice cube tray. The chitosan coating is formed by immersing the blue calcium alginate beads in a chitosan solution, which is prepared by the students from the stock chitosan solution. The blue calcium alginate beads are made fresh, and then incubated for 5 minutes in the chitosan solution. After incubation, each bead is quickly immersed (one by one) in water, just to rinse off the excess of chitosan solution, and then placed in a vial containing distilled water. The solutions are all transparent, so to help students remember where to place the different solutions, a simple scheme is provided (which can be changed as needed). They should use well #2, 4 and 6 as indicated.

The same procedure is followed to prepare the chitosan-coated blue calcium alginate beads and immersed them in a vial containing **milk**. For this part, they should use well #1, 3 and 5 as indicated in the Student Worksheet.

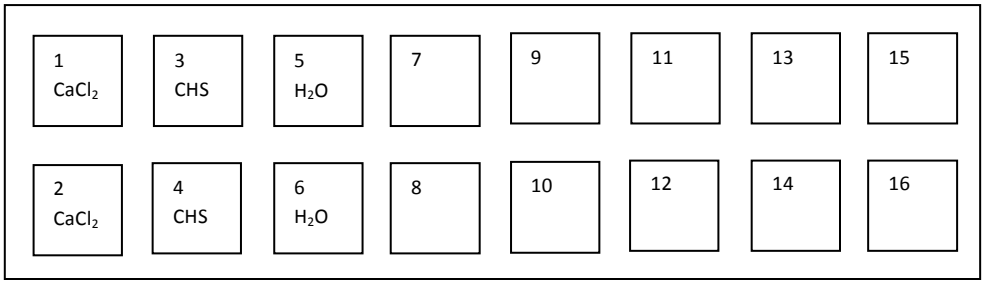

Figure 12. Scheme of the reaction vessels for the coating experiment. Top row can be used for preparing the blue calcium alginate beads, coat them in chitosan (CHS) and immerse them in water. Bottom row can be used for the second test, where beads are made, coated in chitosan and then immersed in milk.

Students make a first batch of blue calcium alginate beads, coat them and then place them in distilled water. Students are asked to make some hypothesis of what the effect of the coating might be, and then should perform the experiment. If they want to make the control (uncoated beads in water) it is suggested they do so after this step.

They will see that the presence of a chitosan shell prevents the release of the dye, even after very long time (overnight). Since the blue dye is released from the calcium-alginate uncoated beads very quickly (even after 15 minutes this effect is seen), there is no need keep the coated blue beads overnight in water to appreciate the effect of the coating. Even after 30 minutes or 1 hour a clear difference is visible. The overnight test is therefore suggested but not strictly necessary to see the difference between uncoated and coated beads in water.

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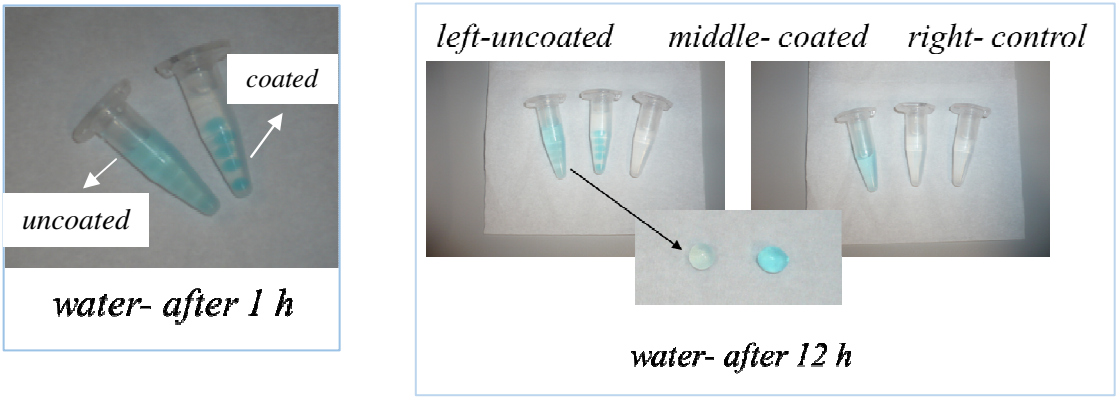

Figure 13. The effect of the "chitosan shell" on the release of the blue dye from calcium alginate beads in water. Box on left: after one hour, the effect of the coating is clearly visible. Box on the right: after 12 hours (overnight test) the coated beads still retain the blue dye and minor release is observed. As indicated in the figure, the vial on the left contains uncoated beads, the one in the middle coated beads, and the one on the right water as control.

The effect of the shell is different when the beads are placed in milk (which is slightly acidic) compared with placing them in water (neutral). Milk also contains enzymes and numerous biomolecules that most likely degrade the chitosan shell. The process in milk is slow, so it is suggested that it is observed after several hours (e.g. after overnight). Figure 14 shows the surnatant after the overnight test: the vial on the left contained uncoated beads, the vial on the middle coated beads, on the right a control vial containing only milk. It can be seen that the dye has been released both from the uncoated and coated beads, although less in the latter case. Students should discuss the result obtained when using water or milk.

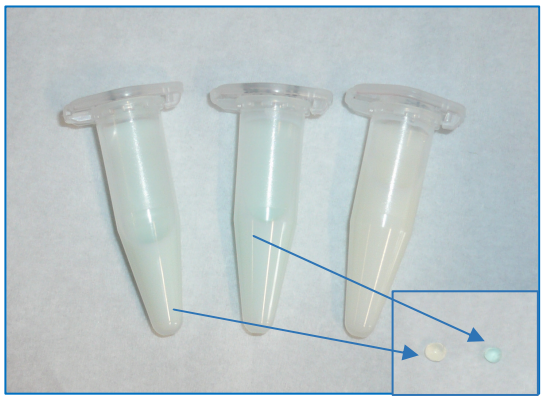

Figure 14. The effect of the "chitosan shell" on the release of the blue dye from calcium alginate beads in milk after an overnight test. Vials contain the supernatant after the overnight test (Left): Supernatant of uncoated beads; (Middle): Supernatant of coated beads; (Right): control (only milk). The inset shows how the blue dye has been released both from the uncoated (left) and right coated bead (right).

The research leading to these results has received funding from the European Community's Seventh Framework Programme (FP7/2007-2013) under Grant Agreement NMP.2011.3-4-290575

It is suggested to use the blue dye alginate beads because the dye diffuses out from the bead very quickly (in 30 minutes) when no shell is added to the bead, so even after a relatively short time, students can see the effect of the chitosan coating. However you might what to ask the students (or a group of them) to try what happens using the red food dye.

Students should complete questions Q12 & Q13 in the Student Worksheet.

REFERENCES & FURTHER READING

1. B.Criswell «Connecting acids and bases with encapsulation...and chemisrty and nanotechnology», Journal of Chemical Education (2007), 84:7, 1136-1139

2. H.G.Bagaria et al., «Self-assembly and nanotechnology: real-time, hands-on, and safe experiments for K-12 students», Journal of Chemical Education (2011), 88, 609-614

3. L. Filipponi, D. Sutherland, "Nanotechnologies, Principles, Applications, Implications and Hands-On Activities, A compendium for educators", 2013, free to download at http://ec.europa.eu/research/industrial_technologies/publications-reports_en.html . See in particular pages: 166-171

4." A step toward minute factories that produce medicine inside the body" http://portal.acs.org/portal/acs/corg/content?_nfpb=true&_pageLabel=PP_ARTICLEMAIN&node_id=223&content_id=CNBP_030456&use_sec=true&sec_url_var=region1&__uuid=d9bfe4ed-47b3-427c-8a8e-a9383841664f

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NANOPINION Educational Resources

Experiment B – Nanoscale thin films

14-18 years

Written by Luisa Filipponi (iNANO)
Interdisciplinary Nanoscience Center
Aarhus University, Denmark
August 2013

MATERIAL INCLUDED IN THIS EXPERIMENT PACKAGE:

For teacher:

TEACHER RESOURCES FOR EXPERIMENT B

For students:

STUDENT LABORATORY WORKSHEET EXPERIMENT B

LEVEL OF EXPERIMENT: **Medium**

DISCLAIMER: The experiments described in the following training kit use chemicals which need to be used according to MSDS specifications and according to specific school safety rules. Personal protection must be taken as indicated. As with all chemicals, use precautions. Solids should not be inhaled and contact with skin, eyes or clothing should be avoided. Wash hands thoroughly after handling. Dispose as indicated. All experiments must be conducted in the presence of an educator trained for science teaching. All experiments will be carried out at your own risk. Aarhus University (iNANO) and the entire NANOPINION consortium assume no liability for damage or consequential losses sustained as a result of the carrying out of the experiments described.

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TEACHER RESOURCES EXPERIMENT B: NANOSCALE THIN FILMS

AIM: Thin films with nanoscale thickness are interesting novel materials that are being investigated in "smart" windows (for instance electrochromic and thermochromic thin films) and in biosensors (for medical detection, food monitoring, etc.). In this experiment students will produce electrochromic thin films of Prussian Blue having different thickness through electrodeposition. To perform the synthesis, they will build a graphite counter electrode. A simple extra laboratory activity (which can be done as a separate, free-standing laboratory activity) is suggested to further demonstrate that this nanomaterial is conductive, as oppose to other allotropes of carbon like diamond. This activity can be used to illustrate a fundamental concept in nanoscience: the nanostructure of a material can affect its properties in unique ways. In the second part of the experiment, the students study the optical properties of the thin films (absorbance) and verify a fundamental law in optics, the dependence between film thickness and magnitude of the absorbance. In the third part of the experiment the students verify the well-known electrochromic properties of Prussian blue thin films.

FIELDS OF NANOTECHNOLOGY APPLICATION: Innovative materials for consumer products; Nanomedicine; Energy

TEACHING GOALS:
- Teach students one of the methods for preparing nanoscale thin films
- Thin film visible light absorbance
- Prussian blue, a material with peculiar optical properties
- Prussian Blue thin films: application to biosensors (medical, food, etc.)
- What is electrochromism, and how it can be used in real-life applications
- Conductivity of graphite

EXTRA TEACHERS' READING: See references in text and "further reading" at the end of the document

REQUIRED STUDENT PRE-KNOWLEDGE: Spectra of light and absorbance; fundamental concepts in electrochemistry; fundamental concepts in electronics (electric circuit, resistance, Ohm law)

The research leading to these results has received funding from the European Community's Seventh Framework Programme (FP7/2007-2013) under Grant Agreement NMP.2011.3-4-290575

BACKGROUND INFORMATION

1. Thin films and their applications

Thin-films are made of layers of materials that can range from fractions of a nanometre (monolayer) to several micrometres in thickness. A familiar application of thin films is the household mirror, which typically has a thin metal coating on the back of a sheet of glass to form a reflective interface. **Nanocoatings** are part of the larger family of thin-films and are defined by their intrinsic functionality, for instance by exploiting the properties of distinct nanoparticles which are embedded in a surrounding matrix, or by their ultra-thin thickness.

Thin films and nanocoatings have several important application in many areas, like medicine, automotive, energy, environment:

- **Tribological coatings**: they play a key role in the performance of internal mechanical components of a vehicle, such as the engine and power train. They are also key elements in cutting tools and machinery in general.

- **Thin films in photovoltaic and batteries**: Thin-film technologies are also being developed as a means of substantially reducing the cost of photovoltaic (PV) systems. The rationale for this is that thin-film modules are cheaper to manufacture owing to their reduced material costs, energy costs, handling costs and capital costs. Thin-film printing technology is being used to apply solid-state lithium polymers to a variety of substrates to create unique batteries for specialized applications. Thin-film batteries can be deposited directly onto chips or chip packages in any shape or size. Flexible batteries can be made by printing onto plastic, thin metal foil, or paper;

- **Responsive coatings**: Responsive nanocoatings are those where the properties of the material in the coating react to environment conditions, such as light or heat, either in a passive or an active way. These coatings allow changing the properties of some materials, such as glass, by conferring new or improved properties. To this group belong a large group of “smart coatings” that include chromic coatings (among which electrochromic;

- **Thin films in biosensors**: Thin films are used in every domain of biosensor systems as for sample handling, biological recognition and amplification, transduction and electronic signal processing.

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2. Prussian Blue thin films

Prussian Blue was discovered in 1704 accidentally by a colour maker named Diesbach of Berlin. Within a short time, it was available for artists to use. It was very popular because previous to its discovery, brilliant, long lasting blues were difficult to obtain. In its 300 year history, Prussian Blue has been used for everything from painting and printing to medicine.

The formula of Prussian Blue is $Fe_4[Fe(CN)_6]_3 \bullet 14H_2O$. The formula is often written $Fe(III)_4[Fe(II)(CN)_6]_3$: Prussian Blue is made up of Fe^{3+}, Fe^{2+} and CN− ions. The $\bullet 14H_2O$ part of the structure refers to 14 "waters of hydration" which are simply water molecules complexed with the compounds. The colour is produced by what is known as metal-to-metal charge transfer. The pigment contains the metal iron (Fe) in two different oxidation states (different ionic forms), Fe^{3+} and Fe^{2+}. In the solid crystal, each of these is coordinated to a CN− ion: the 2+ ion on the carbon end and the 3+ on the nitrogen end: Fe^{2+} – CN – Fe^{3+}

Prussian Blue (PB) can be obtained through classical **chemical synthesis**, leading to a blue colloid, or by **electrochemistry**, giving **a coloured thin film** deposited over a conductive surface. The deposition of PB over a conductive surface is usually carried out from aqueous solutions containing a mixture of ferric (Fe^{3+}) and ferricyanide $[Fe^{III}(CN)_6]^{3-}$ ions, by applying a **reductive electrochemical driving force**. The chemicals normally used are **ferric chloride** ($FeCl_3$) and **potassium ferricyanide(III)** ($K_3[Fe(III)(CN)_6]_3$). The synthesis requires an acidic media, hence diluted HCl is added as well.

3. Prussian Blue optical properties

Thin films of nanometre thickness are studied for many reasons, one being their interesting **optical properties**, which can arise from different phenomena. For instance thin films of titanium oxide with nanometre thickness variations show different colours (for example, a film 100 nm is purple, and a film 30 nm is yellow). The colours are due to the interference of reflected light from the thin transparent oxide[1]. This effect is often illustrated using soap bubbles as an example: the different colours seen are due to the different thickness of the bubbles.

In the case analysed in this experiment, the colour of the thin film is the result of the **absorbance** of Prussian Blue, a material which has a mixture of iron atoms in two oxidation states (Fe^{II} and Fe^{III}) and the colour is produced by what is known as "metal-to-metal charge transfer" effect. It is not in the intention of the author to describe the chemistry of this type of complexes in details, since it would not

[1] If interested in this example, and experiments to demonstrate this in class, see: E. Gaul, Journal of Chemical Education 1993, 70(3), 176-178. Note that a well-equipped electrochemistry laboratory is needed for this experiment.

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be appropriate for the level of the students this experiment is aimed at. It should suffice to explain that in this is a type of complexes, when light is absorbed, a "metal-to-metal charge transfer" occurs, causing and electron to move from the Fe^{2+} ion to the Fe^{3+} though a bridge, in this case a CN− bridge:

$$Fe^{2+} - CN - Fe^{3+} \xrightarrow{\text{light}} Fe^{3+} - CN - Fe^{2+}$$

When this happens, the Fe^{2+} turns into Fe^{3+} due to the loss of an electron. Similarly, the Fe^{3+} gains an electron and thus forms Fe^{2+}. This effectively switches which ion is coordinated to each end of the CN−.

The energy of the photon required is in the **visible range of the spectrum**, hence the bright colour.

FOR YOUNGER STUDENTS: To explain the metal-to-metal charge transfer process, is not necessary to explain the chemical details of Prussian Blue. The process can be explained illustrating Prussian blue as a material that contains atoms of iron with different number of electrons (some have more, some have less), and a "bridge" that allows an electron in an atom of iron with more electrons (Fe^{2+}) to "jump" to an iron atom containing less electrons (Fe^{3+}). The energy to perform this "jump" is given by the incident white light: the energy of the photon required to perform this "jump" is in the visible range of the spectrum (red part), so we see a bright blue colour. The concept is similar to any coloured material, where the colour is due to the absorbance of visible light by a molecule within the material.

The UV-vis spectra of Prussian Blue is shown in **Figure 1** (dotted line), which shows a **pick of absorbance at 690 nm**, in the red region. The figure shows that the colour of the film varies depending on the voltage applied: indeed Prussian Blue is a material that displays electrochromism (and when changes colour, the name of the material changes, e.g., Prussian White etc.). This aspect will be discussed in the next section.

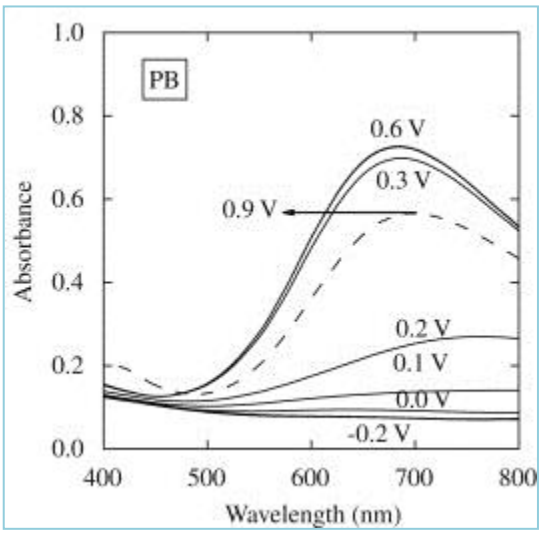

Figure 1. UV-Vis absorbance spectra of Prussian Blue depending on the voltage applied. Reprinted from: Kuo et al., "All-solid-state electrochromic device based on poly(butylviologen), Prussian blue, and succinonitrile", Solar En. Mat. &Solar Cells, 2009, 93, 1755–1760. Copyright Elsevier B.V 2009 all rights reserved.

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Prussian Blue Electrochromisms

Prussian Blue shows **electrochromism**, which has attracted significant scientific attention in research. These thin films of PB are even more attractive due the relation between **electrochemical and optical behaviour** which appears in four-color system, where the colour depends on the various potentials. It is found that PB thin films may be **partially oxidised** to Prussian green (PG) at +0.8 to +0.9 V.

Further oxidation of the partially oxidised PG at +1.2 V leads to **fully oxidised** form known as Prussian brown (PX).

The main application of the PB thin films in the electrochromic devices is based on the electroreduction that yields Prussian white (PW) also known as Everitt's salt (and this is the process we will be focusing on in this experiment). During this process PB thin films become **colourless**. This can be described by the following equation:

$$\underset{\text{PB (blue)}}{[Fe^{III}Fe^{II}(CN)_6]^-} + e^- \rightarrow \underset{\text{PW (transparent)}}{[Fe^{II}Fe^{II}(CN)_6]^{2-}}$$

NOTE! The **electrochromic properties of Prussian Blue depend on the entrapment or release of potassium cations in the crystal lattice**. For this reason the film needs to be immersed in a solution of KCl when performing the electrochromic switch. **Not all cations promote the Prussian Blue/Prussian White electroactivity**. Only ammonium (NH^{4+}), cesium (Cs^+) and Rubidium (Rb^+) were found able to penetrate the Prussian Blue lattice. Other mono or divalent cations are considered as blocking ones (e.g., Na^+).

4. Applications of Prussian Blue thin films

Responsive coatings: "Smart windows"

The use of glass in modern building is very common, since it allows the construction of transparent and seemingly lightweight structures. However the relative high transmittance of visible and infrared (IR) light is a major disadvantage, since this leads to a large heat transfer which is particularly undesirable in summer. The problem is reversed in winter, when heat is dispersed through the glass. In order to address these problems various types of nanocoatings that modulate light transmission in glass are under investigation and commercialised. The aim is to reduce indoor heating in summer, so less air-

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conditioning is required to keep the ambient cool, with consequent energy saving. One type of coating is referred to as “low-e” meaning “low emissivity”, which is based on a thin silver film, about 10nm thick, surrounded by dielectric layers. Metallic layers have been widely used to increase the reflectivity of light (and reduce transmittance) for years, but have the disadvantages of giving a mirror-like appearance. Silver loses its metallic appearance when scaled to a nano-film therefore eliminating this problem. Such a coating is commercialised by Von Ardenne.

“Low-e” coatings are a type of passive nanocoatings, since the properties of the layers are unperturbed during its operation. Another class of coatings used in glasses are those often called dynamic or “smart coatings”. In this case the environmental conditions, such as radiation intensity or temperature, induce a change in the properties of the coating (e.g., darkening of windows). When the effect is a change in the colour (meaning also transparency) they are called “chromogenic smart materials”. The change can be induced actively, by pressing a button. This is the case of electrochromic coatings where applying a small voltage induces a change in transmittance, and in the case of gaschromic coatings, which change their transmittance at the presence of specific gases. Smart coatings can also be passive in the sense of changing their optical properties due to a change of external temperature (thermochromic) or light incidence (photochromic).

The electrochromic properties of Prussian Blue make this material interesting for electrochromic devices. PB thin films are used in electrochromic devices in a *sandwich structure* between two optically transparent conductors. When a negative potential is applied, it yields colourless PW, and when a positive potential is applied oxidation occurs and it yields PB. These thin films are usually combined with thin films of WO_3 (another electrochromic material) in production of electrochromic devices. They are used together in a single device due to the complimentary nature of their electrochromic reactions.

Prussian-Blue based biosensors

Generally speaking, a sensor is a device capable of recognising a specific chemical species and “signalling” the presence, activity or concentration of that species in solution through some chemical change. A “transducer” converts the chemical signal (such as a catalytic activity of a specific biomolecule) into a quantifiable signal (such as a change in colour or intensity) with a defined sensitivity. When the sensing is based on biomolecular recognition it is called a biosensor. There are various types of biosensors, such as antibody/antigen based, nucleic acid based and enzyme based. Also, depending on the technique used in signal transduction, biosensors are classified as optical-detection biosensors electrochemical biosensors, mass-sensitive biosensors and thermal biosensors.

Thin films in sensors and biosensors are used for many reasons, for sample handling, biological recognition and amplification, transduction and electronic signal processing. The thin can be made

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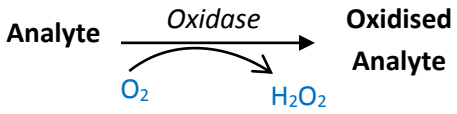
through a number of methods, including layer-by-layer, where specific biomolecules can even be entrapped in the film during its synthesis.

Prussian Blue thin films have been widely used in **amperometric enzyme biosensors**. In these type of sensors the enzyme acts by oxidising the substrate (analyte) and then returning to their original active state by transferring electrons to molecular oxygen, so that the final products are the oxidised form of the substrate (oxidised analyte) and, as a side product, hydrogen peroxide. The analyte is therefore coupled with an enzyme ("enzyme substrate").



The measurement of the oxygen (O_2) consumption and hydrogen peroxide (H_2O_2) production provide information about the initial concentration of the enzyme substrate. In those systems, the use of electrochemical inorganic mediators has shown to reduce the applied potential needed and avoiding several electrochemical interferences. Among those electrochemical inorganic mediators, Prussian Blue have found the largest use, thanks to the reversible reduction and oxidation of this material (Figure 2). In those sensors, the best results (especially for H_2O_2 detection) are obtained when Prussian Blue is deposited in some modified electrodes, like glassy carbon[2].

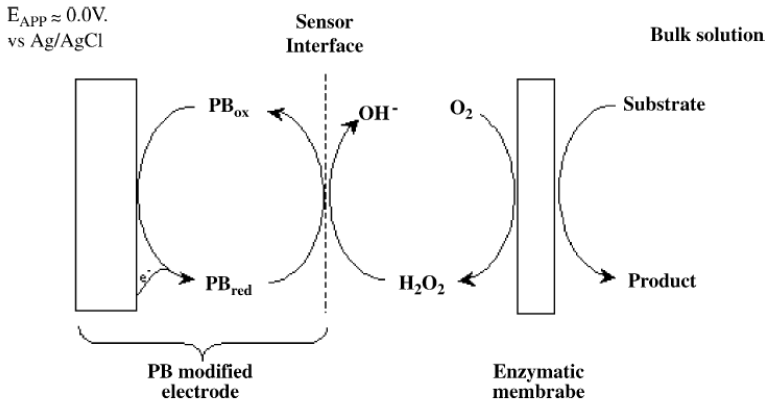


Figure 2. The use of Prussian Blue thin films in amperometric enzyme biosensors. Reprinted from: Ricci et al., "Sensor and biosensor preparation, optimisation and applications of Prussian Blue modified electrodes", Biosensors and Bioelectronics 2005, 21, 389–407.Copyright Elsevier B. V 2005 all rights reserved.

[2] For a good review of Prussian Blue in biosensors, see: Ricci 2005 . The PDF can be retrieved for free using this link: www.francescoriccilab.com/wp-content/uploads/2013/01/12.pdf

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More than ten different enzymes have been coupled with Prussian Blue in amperometric enzyme biosensors for measuring different analytes, for instance glucose oxidase (for **glucose**), cholesterol oxidase (for **cholesterol**), alcohol oxidase (for **ethanol**).

Glucose biosensors based on PB have been successfully applied to whole blood samples. This type of biosensor is very promising for "finger-stick sensors" (disposable) and continuous glucose monitoring, which are both very important for **diabetes monitoring and treatment**.

In the **food industry**, amperometric enzyme biosensors coupled with PB have been shown to be useful for monitoring food spoilage (e.g., production of ethanol) and food quality (for instance glucose is monitored in wine and beverages). Prussian blue amperometric enzyme biosensors have been made also using two or three different type of enzymes: for instance one sensor uses choline oxidase and cholinesterase for detection of pesticides in grape juices.

The optical properties of PB make this material also interesting as **optical transducer in biosensors**. In this case, the detection of H_2O_2 (but also other oxidants) is performed by relating the change of PB film absorbance to the concentration of the analyte. Also, pH changes can be measured with PB thin films, as the film switches from blue to transparent as the pH increases.

OVERVIEW OF THE EXPERIMENT

1. Fabrication of a nanoscale Prussian Blue thin film

The first part of the experiment consists in the **preparation of a thin film with nanometre thickness**. There are numerous methods that researchers use in their laboratories to prepare thin films, for instance spin coating, sputtering, and dip-coating. Under specific conditions, and using some specific instrumentation, these methods allow the formation of thin films with controlled morphology, chemistry and thickness. In a school laboratory those instruments are not readily available; therefore it is suggested to prepare the thin film by **electrodeposition**, using a simple electrochemistry cell designed for this experiment. The advantage of this method is that it allows obtaining thin films with controlled film thickness just by controlling the synthesis time. As such, electrochemistry is interdisciplinary; hence it combines fundamental aspects of chemistry (red-ox reactions, etc.) and physics (Ohm law, etc.).

In this experiment students will prepare **nanoscale thin films of Prussian Blue**, a well know electrochromic material.

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ALTERNATIVE PREPARATION OF ELECTROCHROMIC THIN FILMS

The electrodeposition procedure described in this protocol to create the electrochromic thin film is simple and can be easily replicated in a school laboratory. It uses electrodeposition; therefore some basic electrosynthesis tools are needed. However, if teachers prefer a different fabrication method, one possibility is to make an electrochromic thin film though layer-by-layer assembly, using manual dip-coating. This requires using different chemicals from those described in this protocol, namely a polycation (polyaniline, PANi) and a polycation (sulfonated polysterene, SPS). This protocol requires organic solvents, longer preparation time and uses more expensive chemicals; however the preparation only requires manual deep coating instead of electrochemistry. If interested in this preparation method, teachers should refer to: Schmidt et al., "Layer-by-layer assembly of a pH-responsive and electrochromic thin film", Journal of Chemical Education 2010, 87(2), pp.208-211.

As discussed in the introduction, Prussian Blue can be deposited as a **thin film over a conductive surface by electrodeposition** using an aqueous solutions of **ferric chloride** ($FeCl_3$) and **potassium ferricyanide(III)** ($K_3[Fe(III)(CN)_6]_3$). The synthesis requires an acidic media, hence diluted HCl is added as well.

During the deposition, $K_3[Fe(III)(CN)_6]_3$ is *reduced* at a glass electrode to produce potassium ferrocyanide ($K_4[Fe(II)(CN)_6]_3$) . The $K_4[Fe(II)(CN)_6]_3$ at the glass electrode then reacts with the $Fe(III)Cl_3$ in solution to give insoluble Prussian Blue, $Fe_4[Fe(CN)_6]_3$.

Cell set up and electrodeposition

During any electrochemical synthesis is important to consider safety aspects and communicate them to students: electrochemistry uses currents, and no synthesis should be performed without wearing gloves, or in an open system (e.g. a beaker with immersed electrodes). This is important especially when performing a school laboratory demonstration, since **"laboratory safety " is an educational concept essential not only for ensuring the safety of the students while doing the experiment at school, but also to educate them on some fundamental, good practice actions that a scientists should always perform, regardless of how experienced he or she is**.

In professional laboratories, an electrochemical cell like the one shown in Figure 3 (left) is normally used. This is a closed system with some mobile caps that ensure a way out to gas in case they are formed during the synthesis. However, these types of cells are custom-made and fairly expensive;

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therefore a cheaper alternative is suggested. Figure 3 (right) shows the materials needed and the cell once it is set up:

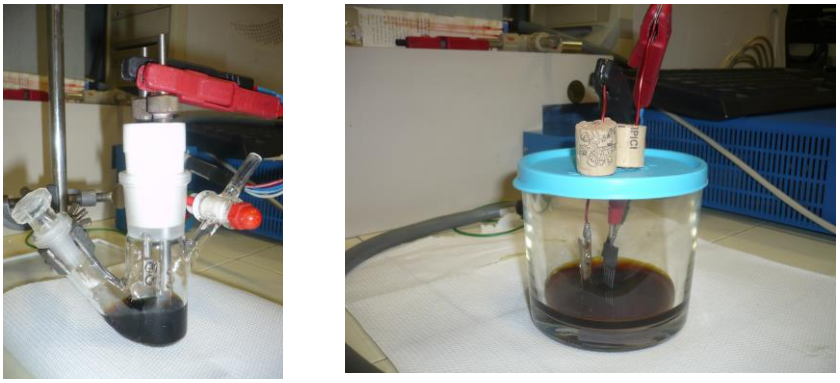

Figure 3. (Left): An electrochemical cell used in professional laboratories; (Right): a simplified electrochemical cell proposed in this protocol.

The food container that holds the liquid has a **plastic lid**- this is important, and food containers with a metal lid should not be used for obvious reasons. Two small holes are made on the plastic lid using a cutter, and the wires holding the electrodes are inserted through these. A third small hole is made (not visible in the image) for safety reason: if hydrogen is produced (water hydrolysis) the gas has a way out of the system. This is a situation not likely to occur but it is a good safety practice example.

The **solution** can be made directly inside the food container by adding the chemicals and gently mix them (see "Procedure" for step-by-step protocol); however it should be made fresh and used immediately.

The **working electrode** is a piece of conductive glass, such as ITO[3] glass. This material normally comes in slides the size of a microscope slide, and needs to be cut in smaller pieces, which is simple accomplished using a diamond cutter. The size of the ITO-glass working electrodes should be approximately 2x1 cm.

A **cheaper alternative to glass-ITO electrode is ITO coated polyester film (PET-ITO)**, which is sold by large chemical companies like Sigma-Aldrich. The advantage of this material is that it can be cut using scissors. However, it is the author experience that the film grown on this material are less uniform, due to different wettability and less uniform ITO coating.

[3] ITO stands for Indium tin oxide; ITO is one of the most widely used transparent conducting oxides because of its electrical conductivity and optical transparency.

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The **counter electrode** is made of graphite (platinum works even better, but it is assumed schools don't have a Pt electrode, or cannot afford to purchase one; however if you do have this, you can use it). A simple way to create a graphite electrode is provided in this protocol (see "Procedure").

The synthesis is performed galvanostatically (constant current) at **40 µA/cm²** using a **galvanostat**. If you don't have one, you can build a device to deposit the current, as reported by other educational groups (see **Appendix II**). The time of synthesis is directly correlated with the thickness of the film produced. It was calculated in previous works that **0.88 nm/sec** of PB is deposited on the working electrode[4].

In this experiment students will create thin films of Prussian Blue having thickness between 25 and 130 nm approximately, as illustrated in the table below:

SAMPLE NAME	DEPOSITION TIME (seconds)	ESTIMATED THICKNESS (nm)
SAMPLE 1	30	26
SAMPLE 2	60	53
SAMPLE 3	90	79
SAMPLE 4	150	132

Table 1

More samples can be made as needed, or with different synthesis times, as chosen by the teacher and class.

PARALLEL ACTIVITY: WHY IS GRAPHITE CONDUCTIVE?

Carbon in nature exists in various forms, all made of carbon atoms, which have very different chemical and physical properties due to the way the carbon atoms are bond together. These different forms are called allotropes, and in the case of carbon include graphite, diamond, and some novel nanomaterials like carbon nanotubes and fullerenes. Graphite is an allotrope that conducts electricity (as opposed to diamond, which does not), and this property is directly connected to its nanostructure. Thus graphite is a simple material that can be used to illustrate a fundamental nanoscience concept: the nanostructure of a material can affect its properties in unique ways. **The conductive properties of graphite can be studied though a simple experiment illustrated at the end of this protocol in a section called "Additional parallel experiment" in APPENDIX 1**.

[4] For the formula leading to this result, see: http://chemistry.beloit.edu/edetc/nanolab/prussianblue/index.html

The research leading to these results has received funding from the European Community's Seventh Framework Programme (FP7/2007-2013) under Grant Agreement NMP.2011.3-4-290575

2. Prussian Blue thin film absorbance measures

In the second part of the experiment, students will study the **absorbance of Prussian Blue nanoscale thin films** they have produced. The absorbance of a coloured thin film at a given wavelength is expected to increase in magnitude as the film becomes thicker (Lambert Law). For instance **Figure 4** shows the absorbance spectra of a series of electrochromic thin films made with a layer-by-layer method, with increasing number of layers (hence increasing thickness).

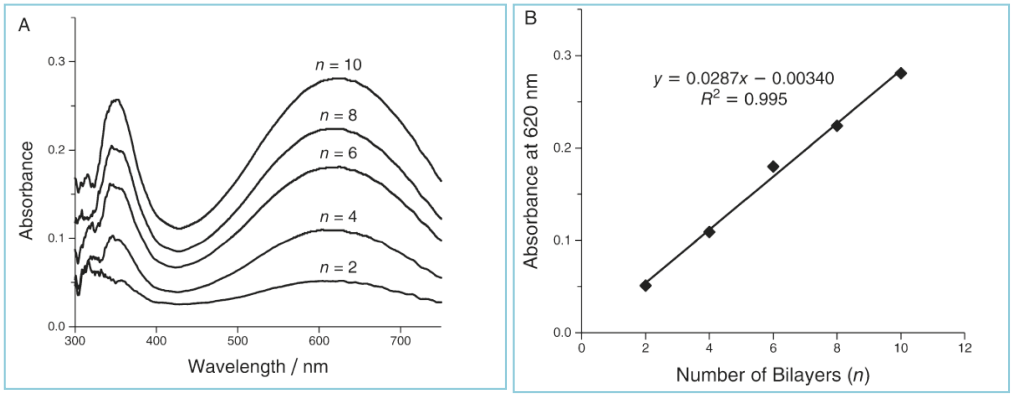

Figure 4. The absorbance spectra of a series of electrochromic thin films made with a layer-by-layer method (left) and the correlation between film thickness and absorbance at 620 nm (peak of absorbance for this material). (Image credit: Reprinted with permission from: Schmidt et al. "Layer-by-Layer Assembly of a pH-Responsive and Electrochromic Thin Film", J. Chem. Edu. 2010, 87(2), 207-211).

In this part of the experiment students will verify this is the case for the Prussian Blue thin films they have prepared.

Absorbance measures using digital colour image analysis

In science laboratories, absorbance measures are routinely performed using a UV-VIS spectrophotometer. This instrument can measure the absorbance of liquids, using a holder called "cuvette" made of quartz or glass (only for visible measures). For thin film, more sophisticated instruments are used. Unfortunately such instruments are not available in school laboratories. Over the last years, some alternative tools have been developed to allow performing absorbance measurements in school, using readily available tools, and using digital colour analysis to extrapolate this information. In the case of liquids, for instance Alexander Scheeline and Kathleen Kelley from the Department of Chemistry of the University of Illinois at Urbana-Champaign have developed diffraction spectrograph/cell phone (or digital camera) array detector suitable for high school and college students. This tool uses a diffraction gradient to split the light coming out from a coloured liquid sample that has been illuminated with a white light. A picture of the spectra is taken using a conventional digital camera,

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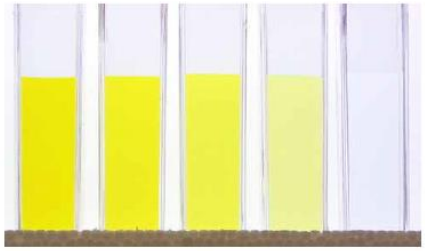
and the image is then analysed using software called "Cell Phone Spectrometer" which elaborates the pictures of the spectra producing in output the intensity, absorption and transmission versus light wavelength plots, according to "absorption spectroscopy" principles[5].

Another method reported in several educational refereed articles is the use of a **desktop scanner** as a way to obtain transparency images of coloured solutions, which can be then analysed using conventional image analysis software. These works have demonstrated that is possible to simulate the visible spectra of coloured samples from the RGB values of its digital colour image (which give the colour intensities of red, green, and blue for pixels within the selected area in the image). This way it is possible to perform colorimetric analysis in school without a spectrophotometer. An example of this approach is illustrated in the work of Kohl and co-workers, who have done a colorimetric assay of yellow dye solution with different concentrations. They collected the digital transparency image of the yellow solutions in a flat bottom transparent plastic microliter plate (**Figure 5**, left) and then by image analysis they collected the intensity of the complementary colour (blue) for each solution (i.e., value "B" in the RGB values of the digital image). The intensity of the complementary colour (blue) decreased linearly with the concentration of the food colouring, resulting in a linear absorbance curve, in accordance with the principle of the Beer-Lambert law. The same approach is reported by Mathews and co-workers for quantitatively measure starch from potato by colorimetric analysis.



Concentration (%)	Red	Green	Blue
0	194	215	192
25	196	219	95
50	197	220	44
75	199	216	22
100	196	211	10

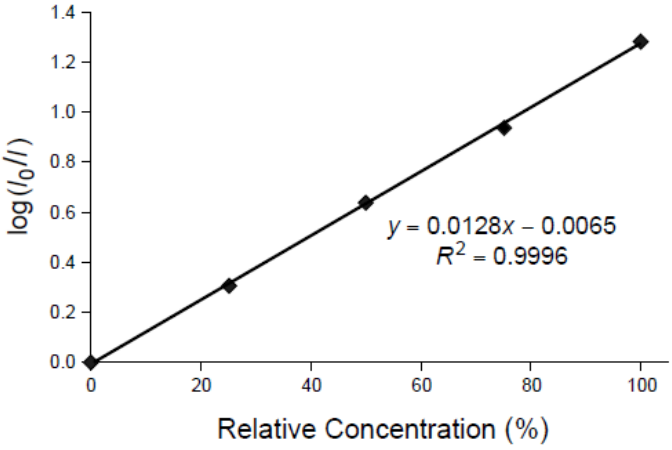


Figure 5 (Top left image): Contrast-enhanced grayscale version of the camera image used for data analysis. Left to right: 100%, 75%, 50%, 25%, 0% relative concentration; (Table below): Image analysis from solutions shown in

[5] For details see: http://www.asdlib.org/onlineArticles/elabware/Scheeline_Kelly_Spectrophotometer/

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the top left image. Data are the average values for R, G, and B (rounded to the nearest integer) of area (100 pixels) at approximately center width and center height of the fluid level of each cuvette. Absorbance plot for blue light intensity by image analysis for the Top left image : I_0 is the blue light intensity for the 0% solution and I is the blue light intensity for the series of solutions. (Image credit: Reprinted with permission from: Kohl et al., "Demonstration of Absorbance Using Digital Color Image Analysis and Colored Solutions", J.Chem Edu. 2006, 83(4), 644-646).

In this experiment students will analyse the **absorbance of coloured thin films**. A coloured thin film is clearly very different from a homogenous coloured solution, especially a thin film that is electrodeposited like in this case (surface roughness is influenced by the quality of the ITO layer over which the film is grown, just to mention one aspect that can lead to film defects). Therefore it is important to recognize than any instrument and method, beside those created for this purpose, will provide an approximate mean of studying the absorbance properties of a thin film, which can be used to verify in class the Lambert Law (i.e., that absorbance is directly proportional with film thickness). Here it is suggested to use the desktop scanner method because it allows collecting information with an acceptable level of approximation for a school level experiment, and it uses an instrument that schools already have.

Transparency scanner images analysis

After producing five samples with different thickness, a desktop scanner is used to collect a transparency image (300 dpi, JPEG).

Figure 6. Four samples of Prussian Blue thin films, grown electrochemically over an ITO glass. First on the left is a piece of ITO with no film deposited. (Image credit: L. Filipponi, iNANO, Aarhus University, Creative Commons Share Alike Non-Commercial 3.0)

The image software used is **ImageJ** (free-ware). Other software can also be used. The JPEG image is opened in ImageJ, an area in the image is selected, and it is used to collect the light intensities (integer 0-255) for the red, green and blue components for pixels within a selected area (100 to 200 pixels is fine). To get this information from ImageJ: Analyze → Histogram

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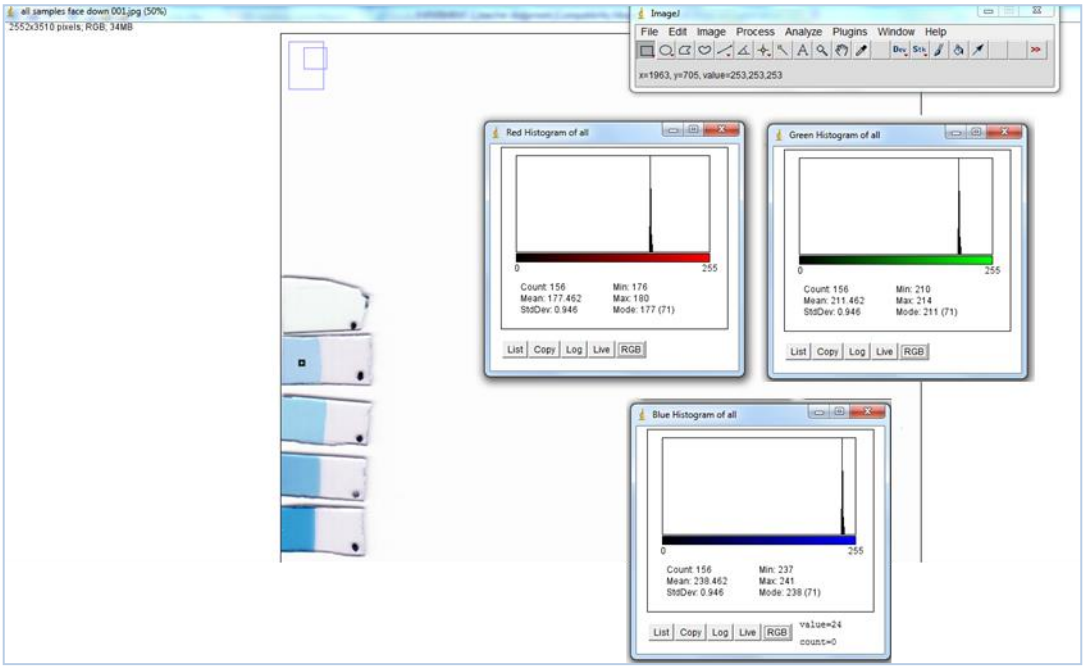

Figure 7. Use of the image software ImageJ to collects R, G and B values of the different thin films. (Image credit: L. Filipponi, iNANO, Aarhus University, Creative Commons Share Alike Non-Commercial 3.0)

The RGB data are collected for each sample, using the same area (with cursor, move area from one sample to the other in the digital image), and the R, G and B values are plotted versus time of synthesis (i.e., 30, 60, 90 and 150 seconds). This allows seeing that the greatest variation is obtained for the Red intensity.

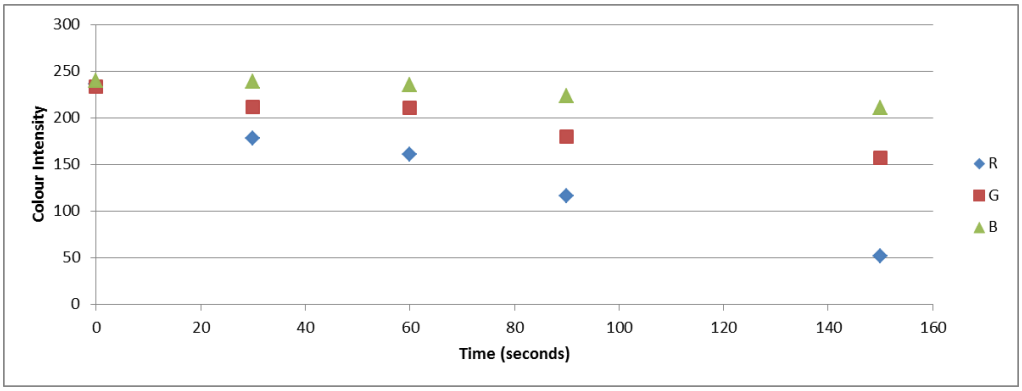

Figure 8. Plot of the R, G and B values are plotted versus time of synthesis (i.e., 30, 60, 90 and 150 seconds) for the samples shown in Figure 6. (Image credit: L. Filipponi, iNANO, Aarhus University, Creative Commons Share Alike Non-Commercial 3.0)

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The image analysis can be used to study the absorbance of the different coloured thin films. Students can use this method to verify that the absorbance increases with film thickness. Prussian Blue has a maximum absorbance peak at 690 nm, as seen in Figure 1, which is in the red part of the visual spectra. However the complementary colour of blue (in digital image analysis) is a combination of red and green, hence we decided to correlate how the sum of the red and green intensity varies with time.
Therefore in the method herein suggested (which is approximate by definition) the absorbance of the PB thin film in its peak region (around 690nm) is studied by analysing the variation of the red plus green intensity values:

$A = \log (I_0/I)$

where

$\log (I_0/I)$ $\rightarrow$ where **I_0** is the "Red+Green" light intensity by image analysis of ITO (no coating) and **I** is the "Red+Green" light intensity by image analysis of the sample with a given thickness.

NOTE! Values of the "blank" (i.e., ITO uncoated) should be collected in an area of the sample where no film was grown. As it can be seen in Figure GG, ITO changes slightly colour (become a bit grey) as a current is passed through it, hence using an un-used ITO would not be appropriate.

Table 2 shows an example on the data collected for the image shown in Figure HH. The RGB values for each sample are collected using the same area (small yellow box in Figure HH), which is moved with the cursor.

	ITO	SAMPLE 1	SAMPLE 2	SAMPLE 3	SAMPLE 4
R	235.71	176.76	158.05	116.36	50.78
G	232.71	210.59	208.92	178.87	155.02
B	239.71	237.65	233.52	221.74	211.15
TIME (SECONDS)	0	30	60	90	150
R+G	468.42	387.35	366.97	295.23	205.8
log[ITO(R+G)]/[R+G]	0	0.082531869	0.106004868	0.200474943	0.357190059

Table 2

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When working with thin films, absorbance is studied against film thickness[6]. In this case, film thickness is proportional to the time of the synthesis, as described before in Table 1. Figure 9 shows the absorbance plot for "red+green" light intensity by image analysis of Figure GG.

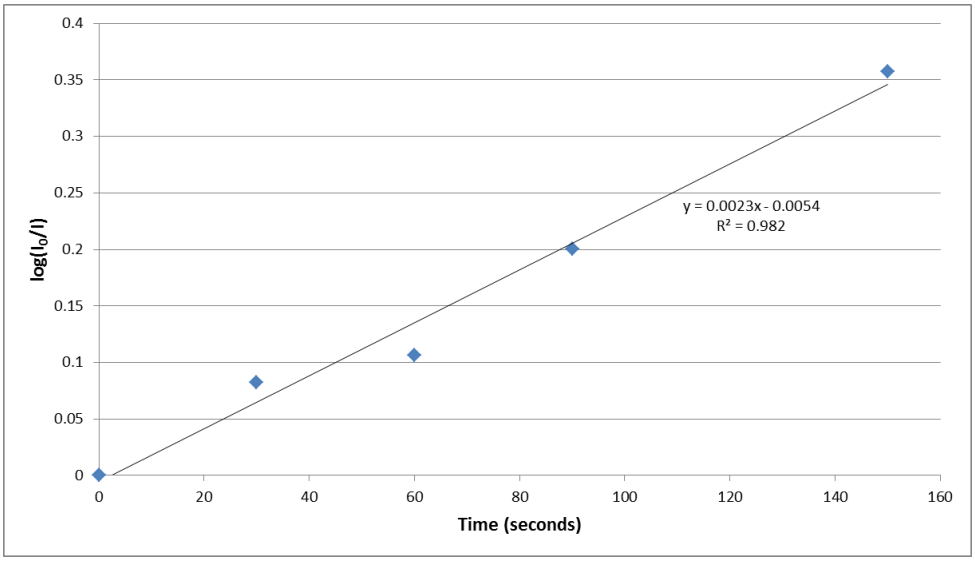

Figure 9. absorbance plot for "red+green" light intensity by image analysis of Figure 6, using data reported in Table 2.

ADDITIONAL SUGGESTED DATA ANALYSIS: The data analysis illustrated should be performed in different regions of each sample, to assess how precise this method is. In addition, a standard deviation could be calculated and used as an error bar for the intensity data, to confirm the validity of the linear regression equation.

[6] Classically, the Beer–Lambert law was first devised independently, where Lambert's law stated that absorbance is directly proportional to the thickness of the sample, and Beer's law stated that absorbance is proportional to the concentration of the sample. The modern derivation of the Beer–Lambert law combines the two laws and correlates the absorbance to both, the concentration as well as the thickness (path length) of the sample. In this experiment, students use the Lambert's Law

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3. Testing Prussian Blue electrochromic properties

As described in the introduction Prussian Blue shows electrochromism. In this part of the experiment, students will use a PB thin film prepared in the previous part of the experiment and verify this electrochromic property, specifically the electroreduction to Prussian white (WP): they will apply a positive voltage using a 1.5 V battery to the film (while this is in a KCl bath) and see the **colour variation from blue to transparent**. The process is reversible, and this can be seen by bringing the voltage to zero, by connecting the glass electrode directly to the graphite electrode.

THIS EXPERIMENT IN CLASS

The amount of materials, chemicals and equipment needed to run this experiment depends on how the teacher plans on performing it, and mostly on how many preparation and analysis "workstations" he/she can set up for the class. This experiment can be run in class dividing the students in group of two, and then each group can be given a "workstation" with the tools to prepare the Prussian Blue thin film samples, and test them for electrochromism. Alternatively, if equipment is limited, the teacher can set up one "workstation" and ask the different groups to take turns preparing the samples (e.g., each group makes a sample with a different thickness) and takes turns in testing them. This is the cheapest option (one galvanostat needed) and safer option (teacher can supervise each group performing the synthesis). Note that an alternative to a potentiostat/galvanostat instrument is provided in Appendix II.

To make the most of this experiment, it is recommended using it together with the two **nanOpinion Moodle minicourses called "Smart Surfaces" and "Smart Food Packagings"**, where students can see some practical applications of different types of surface nanoengineering and nanocoatings (http://nanopinion-edu.eu/).

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MATERIALS AND STOCK SOLUTION

Chemicals (can be all bought from Sigma-Aldrich, www.sigmaaldrich.com/):

NAME	QUANTITY	SIGMA PRODUCT NUMBER/ CAS NUMBER/ APPROX. COST	STOCK SOLUTION
Ferric chloride hexahydrate (also called: Iron(III) chloride hexahydrate): $FeCl_3 \cdot 6H_2O$	100 gr	236489 [10025-77-1] 22 Euro	0.05M (Dissolve 1.35 g in 100 mL of distilled water with a drop of HCl). For each preparation you need 10 mL of the stock solution.
Potassium ferricyanide(III): $K_3Fe(CN)_6$	50 gr	702587 [13746-66-2] 34 Euro	0.05 M (Dissolve 1.65 g in 100 mL distilled water). For each preparation you need 10 mL of the stock solution
Potassium chloride: KCl	It is assumed schools have this reagent; if not see www.sigmaaldrich.com/ for purchase options		1 M (each electrochromic test needs 25 mL; dissolve 18.6 g in 250 mL water with two drops of HCl to lower the pH)
HCl conc. (37%)	It is assumed schools have this reagent; if not see www.sigmaaldrich.com/ for purchase options		0.05 M (Dilute 5 mL conc. HCl to 250 mL in water). For each preparation you need 5 mL of the stock solution.

NB. The volume of the stock solutions should be changed based on the number of preparations you need to perform!

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Other materials needed:

ITO Conductive glass	ITO slides are sold by several companies, including Sigma-Aldrich For this experiment you can use ITO glass with a resistance between 10 and 25 ohm (e.g., Sigma-Aldrich product number 703192, 110 Euro for a pack of 10 square slides 25 mm × 25 mm × 1.1 mm. Slides should be cut in two. Rectangular slides are available but more expensive). An alternative product to use is PET-ITO (e.g., Sigma Aldrich product # 639303, about 20 Euro for a sheet 1ftx 1ft x 5mm), which is cheaper, but has some limitations (less uniform deposition).
Distilled water	1 L
Batteries 1.5 V	Depends on how many "working stations" are set up. Each working station needs one battery 1.5 V
Graphite lead 2mm HB	Depends on how many "working stations" are set up. Each working station needs a graphite reference electrode, which is made using 5 graphite leads. Packs of 10 graphite leads are available at stationary shops.

NOTE! ITO glass can be cleaned and reused by removing the Prussian blue coating with concentrated ammonia (careful!)

LAB EQUIPMENT NEEDED

For the entire class:

Scale with 0.1 g precision
Galvanostat*
Ohmmeter*
A desktop scanner connected to a PC
Glass cutter

* the number of galvanostat depend on how many "working stations" are set up. The ohmmeter can be shared by the entire class. See Appendix II if you don't have a galvanostat.

Materials needed for the electrodeposition:

Several pieces of ITO conductive glass 2x1 cm (the exact number depends on how the experiment is set up in the class)
One graphite electrode (see page 24 for instructions to make one)

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One food container with plastic lid[7] (with a capacity between 50 to 100mL approx.).
One bottle cork
Two metal wires with insulator coating having an alligator clip solded at one end
Electric wires
Galvanostat (or if you don't have one, see Appendix II)
0.05 M Ferric chloride hexahydrate solution $FeCl_3$ (each thin film synthesis needs 10mL[8])
0.05 M Potassium ferricyanide(III) ($K_3Fe(CN)_6$) (each thin film synthesis needs 10mL)
0.05 M HCl ((each thin film synthesis needs 5mL)
Three 25 mL or 50 mL cylinder[9]
A small plastic container (to hold the ITO thin film samples after they are prepared), or closed test tube.
A paper cutter
Tweezers
Gloves
Paper
Eye protection

Materials needed for the electrochromic test in:
The electrochemical cell described above
25mL of the 1.0 M KCl stock solution
One 1.5 V battery
Electric wires
Gloves

SAFETY NOTE: This experiment doesn't use chemicals but only common liquids and solids. Nevertheless staining is possible so wash hands and surfaces thoroughly after handling. Use appropriate clothing protection, gloves and eyes protection. Collect all liquids and washing water is glass/plastic containers and dispose of in sink. All experiments will be carried out at your own risk. Aarhus University (iNANO) and the entire NANOPINION consortium assume no liability for damage or consequential losses sustained as a result of the carrying out of the experiments described.

[7] Prefer tall, rather than shallow, containers. In this protocol, a 70 mL capacity FRIGOVERRE (Bormioli) 12 tall with blue lid has been used.

[8] Smaller or larger volumes should be used depending on volume of container.

[9] Or if not available, use less but rinse in between measurements.

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PROCEDURE

Preliminary setting up and material preparation

1) Before starting the experiment, **ITO-glass samples should be prepared by cutting the ITO-glass** as purchased, aiming at 2x1 cm size samples. Cut the ITO-glass using a glass cutter. Cutting ITO glass slides is not particularly difficult; it is done with a glass cutter and a bit of a gentle touch. You can get some practice on a couple of normal microscope slides. The cutting should be done by the teacher, of course wearing gloves (to protect your fingers and the ITO coating. Pieces of glass-ITO should be cleaned before being used, by washing in water/soap, rinse with distilled water, and final rinse with acetone (if available). Gently dry with paper (don't scratch!), and store in a dry place. Samples should be cleaned shortly before use in electrodeposition.

2) **Find for each ITO-glass sample which is the conductive side.** This part can be done by the students, although it is important that they hold the samples with gloves (to avoid finger prints!). The students than need to decide which is the conductive size of the ITO-glass using an ohmmeter to measure resistance; the conductive side will have a resistance of 12-25 ohm, depending on the product you use.
Mark each sample, for instance with a small dot (**use permanent market**) on right hand corner. Keep cut glass-ITO pieces in a closed vial or beaker to avoid dust.

3) **Estimate the area of deposition**: in this protocol it is assumed that the deposition area will be 1x1 cm. If the area immersed in the solution is different from 1 cm^2, you will need to accordingly adjust the current using for electrodeposition (e.g., 80 µA if area is 2 cm^2). You should **first measure the area of the working electrode that will be immersed** (you can put a line with a marker to define it), **and calculate the current** that should be used to reach 40 $\mu A/cm^2$.

3) Wash with soap/water the food container and rinse well. Air dry.

4) Cut the wine cork in two pieces using a paper cutter

5) **Prepare the graphite counter electrode (**if you don't have a Pt or graphite counter electrode):

A simple way to create a graphite electrode is to use graphite leads HB 2mm in size. To create the graphite counter electrode, simply take five graphite leads: one should be longer and placed in the middle (the alligator will be attached to this lead), and place the remaining four next to it, two on each side. Wrap them together using a conductive tape or conductive paste. If you don't have a conductive tape you can use conventional tape. Then cut the leads at the bottom so they are all the same length.

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See **Figure 10** for the final product. The dimension of your counter electrode should be similar to the size of the working electrode. Before using the graphite electrode polish gently its surface with a mesh. Note that the working and counter electrode should have similar size.

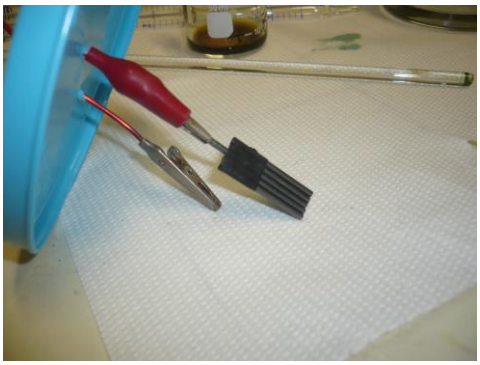

Figure 10. The graphite counter electrode used in this experiment (to be made if your school doesn't have a platinum electrode). (Image credit: L. Filipponi, iNANO, Aarhus University, Creative Commons Share Alike Non-Commercial 3.0).

The number of graphite electrodes you will need to prepare depends on how many electrodeposition "working stations" you will set up.

TIP TO TEACHER: You can use the simple experiment "Parallel activity: graphite conductivity" at the end of this document to further discuss with your students the conductivity properties of graphite (Appendix I).

1. Electrodeposition of Prussian blue thin film

Open the food container and cut the plastic lid with a cutter to create two parallel holes where you will insert the electrodes. Make a third hole somewhere else in the lid (safety hole for letting gases out). Place the two metal wires through the lid where you created the holes, and place the working electrode and counter electrodes using the alligators.

Insert the wine cork inside the wire to add stability to it (see Figure 11). Push the wires upwards so that the electrodes are close to the lid.

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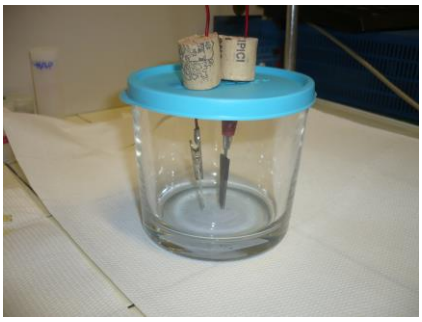
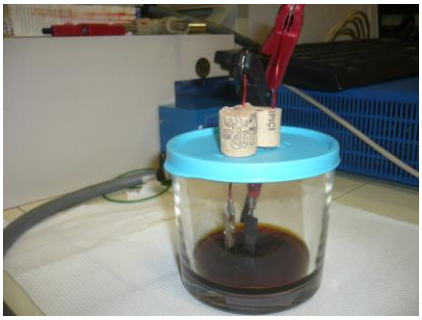


Figure 11. The electrochemical cell once it is set-up and ready to be used. (Image credit: L. Filipponi, iNANO, Aarhus University, Creative Commons Share Alike Non-Commercial 3.0)

Now measure and mix the synthesis solution directly in the glass food container. **NB** Open the food container, remove lid and set aside, add solutions and mix gently with a glass rod or spoon:



10 mL of 0.05 M $K_3[Fe(CN)_6]$
10 mL of 0.05 M $FeCl_3 \cdot 6H_2O$
5 mL of 0.05 M HCl

Assemble the cell by placing the lid with the **working electrode parallel to the counter electrode**. Electrodes should have similar size, and the **electrical contact (i.e., the alligator clips) should not touch the solution.** The electrodes should NOT touch the solution at this stage.

Now push gently the electrodes to touch the solution: you should aim at having an area of 1x1 cm immersed. Connect the working electrode (glass-ITO) to the negative lead of the galvanostat and connect the positive lead to the counter electrode (Pt or graphite).

Start the electrodeposition: the synthesis is performed galvanostatically (constant current) at **40 µA/cm^2**.

Perform the synthesis on different samples for different time. It is suggested to start with a fresh solution at each synthesis (especially for long synthesis time, i.e., over 60 seconds)

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Samples can be prepared as in table below:

SAMPLE NAME	DEPOSITION TIME (seconds)	ESTIMATED THICKNESS (nm)
SAMPLE 1	30	26
SAMPLE 2	60	53
SAMPLE 3	90	79
SAMPLE 4	150	132

After each synthesis, rinse the working electrode with distilled water and place on a piece of paper towel to let it dry. If not used immediately after, store samples in a closed plastic container.

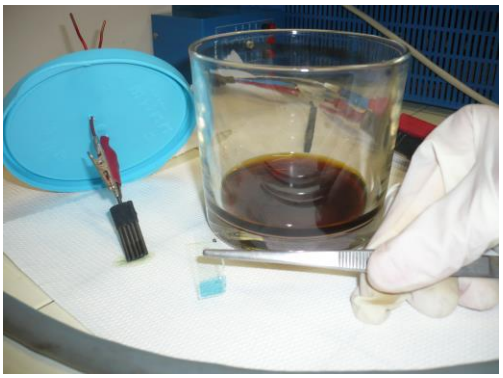


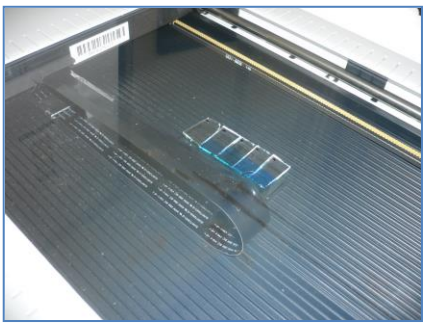
Figure 12. The working electrode after the electrochemical synthesis. (Image credit: L. Filipponi, iNANO, Aarhus University, Creative Commons Share Alike Non-Commercial 3.0).

TIP TO TEACHER: discuss why it is important to have a set up where the distance of the electrodes is kept constant during the electrodeposition. Calculate charge from synthesis time (Q=I*t, where I is the current and t is the time of synthesis).

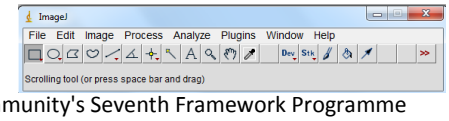
2. Thin film optical properties

In this part of the experiment, students will use an indirect method for obtaining information on the absorbance of the thin films they have produced. It is important to highlight to the students the limitations of the method used.

Download ImageJ on your computer from: http://imagej.en.softonic.com/



Collect a transparency scanner image by placing the samples face-down on the central part of a desktop scanner. Place a piece of white paper over the samples, close the lid of the scanner and scan to collect a JPEG image (300 dpi).



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Open ImageJ and open the image you have just scanned. Using the square selection tool, select an area of approximately 100x100 pixel and collect the RGB data for the selected area (Analyze → Histogram). Repeat the same for the different samples, by moving with the cursor the selection area to the next sample (area should not be changed in size). Aim at the same location in each sample, e.g., middle part. Repeat for all samples, and collect the same information for uncoated ITO.

Values of the "blank" (i.e., ITO uncoated) should be collected in an area of the sample where no film was grown. As it can be seen in Figure GG, ITO changes slightly colour (becomes a bit grey) as a current is passed through it, hence using an un-used glass- ITO would not be appropriate.

Students should collect the RGB data for all samples, and then prepare a table like the following:

	ITO	SAMPLE 1	SAMPLE 2	SAMPLE 3	SAMPLE 4
R	235.71	176.76	158.05	116.36	50.78
G	232.71	210.59	208.92	178.87	155.02
B	239.71	237.65	233.52	221.74	211.15
TIME (SECONDS)	0	30	60	90	150

Table 3. An example of data collection using ImageJ data analysis of scanned images

For each sample, the "R+G" value should be calculated and then the absorbance obtained through the formula:

$A = \log (R+G)_{ITO}/(R+G)_{sample}$

	ITO	SAMPLE 1	SAMPLE 2	SAMPLE 3	SAMPLE 4
R+G	468.42	387.35	366.97	295.23	205.8
log[ITO(R+G)]/[R+G]	0	0.082531869	0.106004868	0.200474943	0.357190059

Next, students should produce a plot "A vs. time" and calculate the regression equation (or A vs. thickness in nm, since thickness=0.88 nm* time of synthesis):

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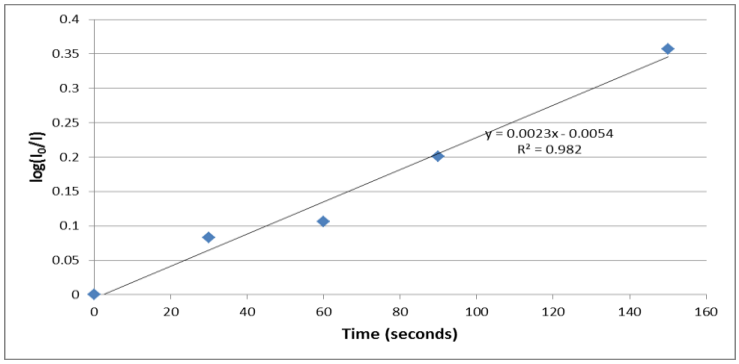

Figure 13. Example of a plot obtained using the desktop scan method and digital image analysis.

In addition to this, students can:

- calculate RGB values on different locations of each sample (to obtain average and standard deviation of data; add error bar on values in **Figure 13**; overall assess the precision of the technique).

3. Test a Prussian blue thin film for electrochromism

In this part of the experiment, students will use the PB thin film they have prepared and study their electroreduction to Prussian white (WP): they will apply a positive voltage using a 1.5 V battery to the film and see the **colour variation from blue to transparent**.

To do so, take a clean food container and insert the wires and electrodes as described in section2. Assemble the working electrode (PB thin film sample) and the counter electrode (Pt, if you have it, or graphite).

Add 25 mL of KCl 1 M to the glass food container and add few droplets of HCl 0.05 M to make the solution acidic. Mix with the glass rod. Gently place the plastic food container lid: electrodes should be parallel and the alligators should not touch the solution. To stabilize the film, it is suggested to first apply a negative voltage to the film: glass electrode to battery (-) and graphite electrode to battery (+). Now bring voltage to zero, by connecting the glass electrode directly to the graphite electrode: the film turns blue. After doing this a couple of times, you can **test the electrochromism from Prussian blue (PB) to Prussian white (PW)**: connect glass electrode to battery (+) and graphite electrode to battery (-): a positive voltage is applied and the film goes from blue to transparent. To switch the film back to blue, bring voltage to zero, by connecting the glass electrode directly to the graphite electrode.

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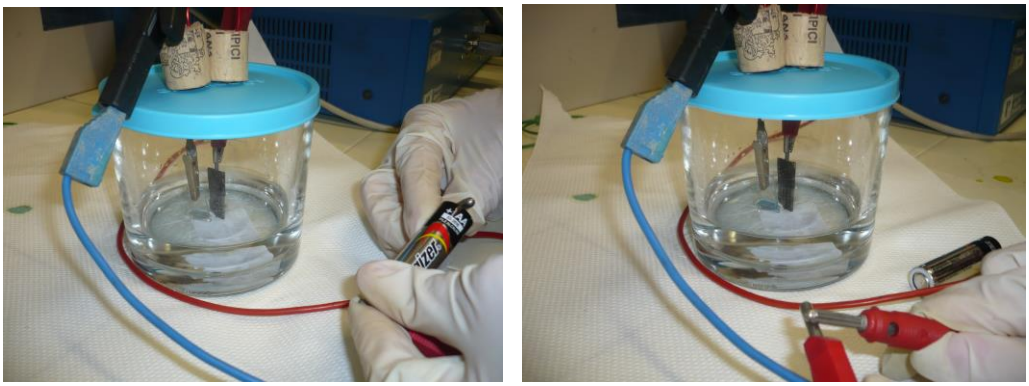

Figure 14. Testing a sample of Prussian Blue thin film for electrochromism. (Image credit: L. Filipponi, iNANO, Aarhus University, Creative Commons Share Alike Non-Commercial 3.0).

APPENDIX I

PARALLEL ACTIVITY: GRAPHITE CONDUCTIVITY

In Nature there are some pure materials that have striking different properties even though they are made of the same atoms. For instance, graphite and diamond (**Figure 15**): two very popular materials, one used conventionally in pencils and the other in jewellery. These two materials could not be more different: graphite is soft, light, flexible, and conducts electricity while diamond is extremely strong, hard and does not conduct electricity. Both materials are made of atoms of carbon linked through strong bindings (covalent), but in graphite each carbon atom uses three out of its four electrons to form single bonds with its neighbours, forming a linear sheet, whereas in diamonds each carbon atom uses all its four electrons to form four single bonds, resulting in a 3-D structure. **The different properties of graphite and diamond are a consequence of the different way the carbon atoms in the materials are bonded together**.

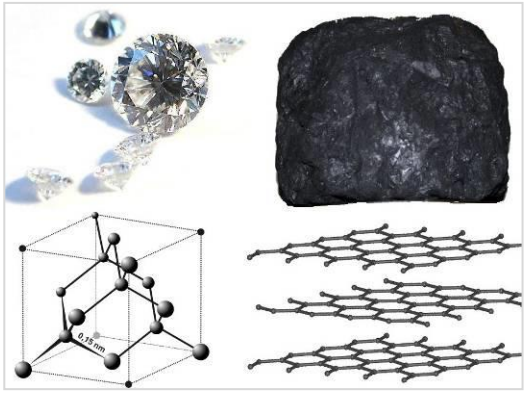

Figure 15. Two allotropes of carbon and their respective chemical structure: (left, top and bottom): diamond; right (top and bottom): graphite.(Image credit: Wiki Commons, Creative Commons Attribution ShareAlike *3.0)*

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Graphite will be well known to students, as it is the material pencils they use are made of. However, in those tools graphite is used because it is a soft material that leaves a mark which can be easily erased (as a matter of fact, the term "graphite" comes from *graphein*, meaning *to write/draw* in Ancient Greek). In this simple activity students will learn that the lead their pencils are made of have also another property: it conducts electricity, and in fact can be used in a circuit to light up a LED.

Why does graphite conduct electricity?

Graphite has a layered, planar structure. In each layer, the carbon atoms are arranged in a honeycomb lattice with separation of 0.142 nm, and the distance between planes is 0.335 nm. Graphite can conduct electricity due to the vast electron delocalization within the carbon layers. These valence electrons are free to move, so are able to conduct electricity. However, the electricity is primarily conducted within the plane of the layers[10]. The use of graphite in batteries has been increasing in the last 30 years. Natural and synthetic graphite are used to construct the anode of all major battery technologies.

THIS EXPERIMENT IN CLASS

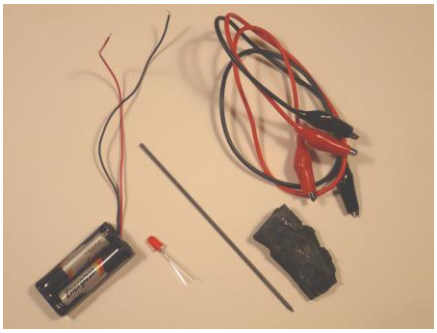
The following is a very simple experiment to show students the **electrical conductivity of graphite**. Some possible teaching goals are:

- Illustrate students a fundamental nanoscience concept: the properties of a material depend on their nanostructure, and materials made of the same type of atoms (allotropes) can have very different properties due to the way the atoms are bond together and the nanostructures they form

- Study some basic principles of electricity (resistance, resistivity, Ohm law)

MATERIAL NEEDED

Two AAA 1.5 V Batteries
Battery holder with connecting wires
Two connecting wires with alligators on their ends
LED (20 mA, 2.9 to 4.2 V, violet, pink, purple or white LED)
Pencil lead
A piece of charcoal



[10] http://en.wikipedia.org/wiki/Graphite

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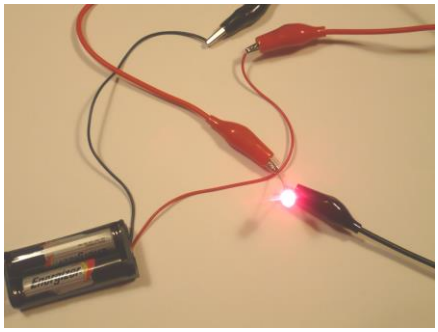
PROCEDURE

1) Connect battery to LED using wires (Figure VC): the LED will light up. **NB**. The LED is directional, and the longer metal stem in it should be connected to the positive pole of the battery.



2) Now introduce the graphite lead in the circuit to verify it conducts electricity. Connect the battery to the graphite, the other end of the graphite to the LED, and the LED to the battery (the circuit is now closed), as shown in **Figure 16**. The LED will light up.

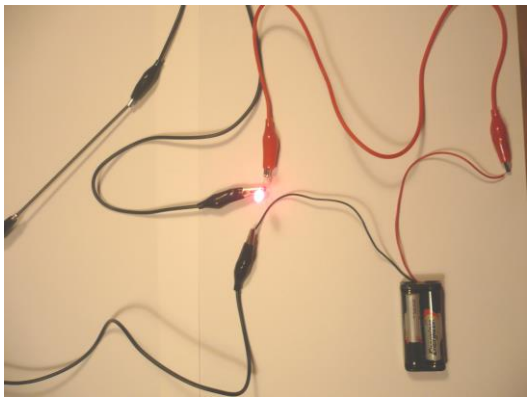


Figure 16. A graphite lead is inserted in the circuit to verify it conducts electricity. (Image credit: L. Filipponi, iNANO, Aarhus University, Creative Commons Share Alike Non-Commercial 3.0).

3) To show students that not all forms of carbon conduct electricity, repeat the test # 2 using a piece of charcoal instead of graphite: the LED will not light up (Figure 17).

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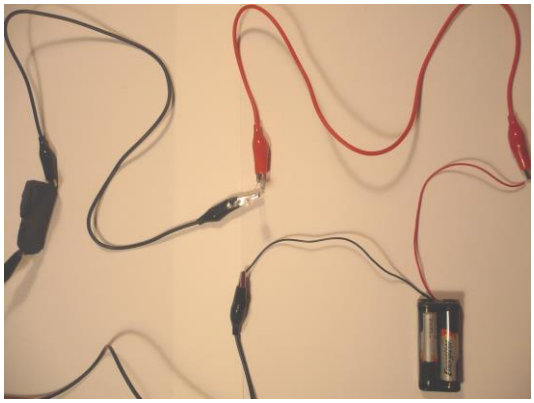

Figure 17. When a piece of charcoal is inserted in the circuit, the LED does not light up, confirming it does not conduct electricity. (Image credit: L. Filipponi, iNANO, Aarhus University, Creative Commons Share Alike Non-Commercial 3.0).

A diamond would also give the same result: point out this to your students!

4) Discuss with students why they think graphite conducts electricity and charcoal or diamond not, being those materials made of the same type of atoms. You can show images of the nanostructure of graphite and diamond as they make hypothesis (**Figure 15**) and discuss their results. If you use in teaching an enquiry-based approach, you can collect students' hypothesis before running the experiment, without giving students any pre-knowledge, and do an after-experiment discussion to reach conclusions on the properties of graphite.

APPENDIX II

A galvanostat is an instrument to provide a constant current to an electrochemical cell[11]. Often it comes as a potentiostat/galvanostat, meaning it can either keep the current constant or the voltage constant. The instrument is fairly simple to use; however, **if the school doesn't have one, for this specific experiment, a simple version can be "assembled"** by using a 1.5 Volt battery, a holder, and 50 K ohm variable resistor for controlling deposition current. This set up has been reported in some online educational material (http://education.mrsec.wisc.edu/291.htm) however it was not tested by the author of this document.

[11] https://en.wikipedia.org/wiki/Galvanostat

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The diagram and picture of this set-up is provided in **Figure 18** in next page. If teachers decide to use this home-built device, it is still recommended to set up a closed electrochemical cell, like the one illustrated in the teacher guide.

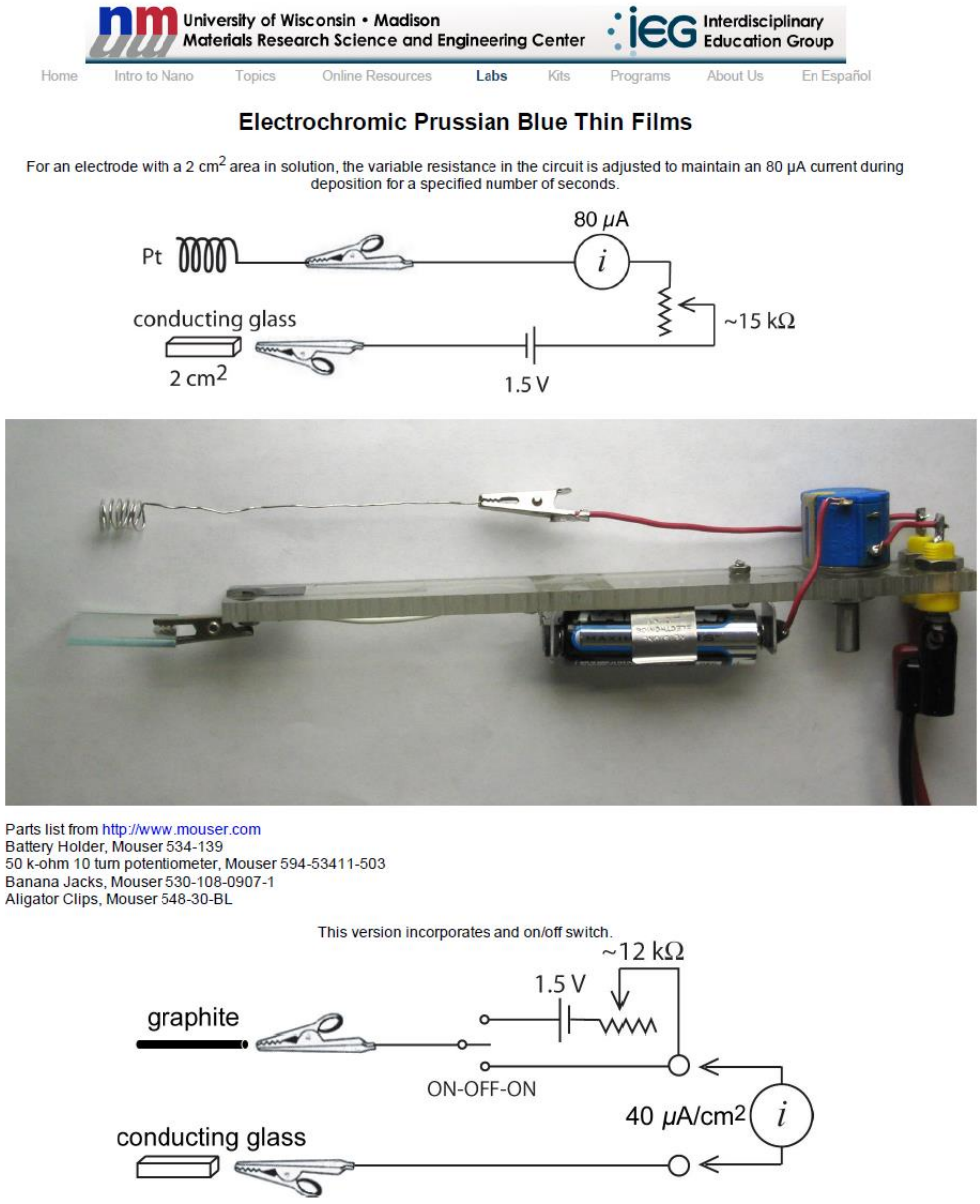

Figure 18. Set up of a simple device for delivering a constant current to the working electrode (ITO glass) as alternative to the use of a galvanostat. (Image credit: Image kindly provided by MRSEC Education Group, University of Wisconsin-Madison).

The research leading to these results has received funding from the European Community's Seventh Framework Programme (FP7/2007-2013) under Grant Agreement NMP.2011.3-4-290575

CREDITS

This experiment herein reported has been partly adapted from the experiment “Electrochromic Prussian Blue Thin Films”, MRSEC Education Group, University of Wisconsin-Madison, http://education.mrsec.wisc.edu/291.htm

The electrochemical synthesis reported in this experiment has been conducted in the Electrochemistry laboratory of Professor Stefania Panero, University of Rome- La Sapienza. The author wishes to thank the collaboration of Dr Judith Serra Moreno for running the experiment and collect data for its analysis.

The author wishes also to thank Dr Alexander Prokop for reviewing this experiment and for providing fruitful comments during its finalization.

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- Schmidt et al. “Layer-by-Layer Assembly of a pH-Responsive and Electrochromic Thin Film”, J. Chem. Edu. 2010, 87(2), 207-211).

For an introduction on nanocoatings and their different applications, see:

- L. Filipponi, D. Sutherland, "Nanotechnologies, Principles, Applications, Implications and Hands-On Activities, A compendium for educators", 2013, free to download at http://ec.europa.eu/research/industrial_technologies/publications-reports_en.html. See in particular pages: 119-122; 157-163; 187-192; 197-198

For an introduction on graphite, graphene and other carbon allotropes:

- L. Filipponi, D. Sutherland, "Nanotechnologies, Principles, Applications, Implications and Hands-On Activities, A compendium for educators", 2013, free to download at http://ec.europa.eu/research/industrial_technologies/publications-reports_en.html. See in particular pages: 106-111

- Nobel Prize in Physics 2010, Advanced Information, http://www.nobelprize.org/nobel_prizes/physics/laureates/2010/advanced.html

The research leading to these results has received funding from the European Community's Seventh Framework Programme (FP7/2007-2013) under Grant Agreement NMP.2011.3-4-290575