# STEM Teaching Kit on Testing and Certification for Junior Secondary Students

**Student Laboratory Manual** 

Department of Chemistry Hong Kong Baptist University

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# **Important Note**

Students must read the **safety precaution** of each experiment in this Manual carefully beforehand and take all necessary safety precautions in conducting the experiments carefully. Advice and information offered in this Manual are by no means exhaustive and do not preclude the need for exercising care and good judgement at all times in safeguarding against accidents. When in doubts, please seek instructions from your teachers immediately.

## Module 1 – Analysis of Colorants in Food and Personal-Care Products

#### Introduction

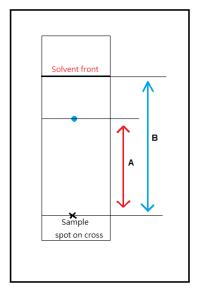
Colorant is a substance which is added to change the color of a material. The addition of colorants in food makes the food products more attractive and appetizing, while the use of colorant in personal-care products is mainly for decorative purpose.

Colorant can be either natural or synthetically made compounds. For example, carotenes are an example of natural colorant because it is obtained from carrot. However, most of the natural colorants are unstable and easily degraded by light and temperature. Hence, the use of synthetic colorants is more popular. This is because the synthetic colorants are more soluble in hydro-/oil-based consumer products and relatively low price. Nowadays, both natural and synthetic colorants can be found in food products, while the synthetic ones are more commonly used in the production of personal-care products.

Recently, there are lots of concerns about the safety of using synthetic colorants because these chemicals are heavily linked with cancers. Since the colorants are colored, paper chromatography is an ideal analytical tool to detect it in food and personal-care products.

#### What is paper chromatography?

Paper chromatography (PC) is an analytical method based on the principle of partition separation. It is fast and requires small amount of sample. The sample is first held in the pores of filter paper and then travels together with the developing solution on the filter paper. Depending on the nature of the chemicals, they travel in varied speed on the filter paper. By measuring the distances of the sample and solvent travelled, retention factor (R<sub>f</sub>) of each component can be calculated (Figure 1).



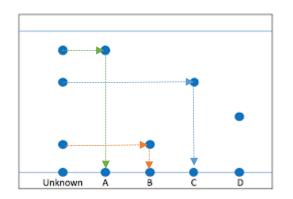
$$R_{f}$$

$$= \frac{Distance moved by the sample}{Distance moved by the solvent}$$

$$= \frac{A}{B}$$

Figure 1. Calculation of R<sub>f</sub> value.

By comparing the R<sub>f</sub> values of extracted colorants from samples with the ones of known chemical standards OR simply the final positions of each spot, the unknown components in the sample can be identified (Figure 2).



**Figure 2.** R<sub>f</sub> value comparison, indicating the unknown contains chemicals A, B, and C.

#### **Intended Learning Outcomes**

After the activity, the student is expected to be able to:

- 1. understand the importance of testing services in assuring the quality and safety of food and personal-care products, and the role played by testing in daily life;
- 2. set up a paper chromatography using simple apparatuses;
- 3. analyze colorants in food and personal-care products by paper chromatography;
- 4. acquire the basic concepts of STEM.

#### Experiment

Apparatus

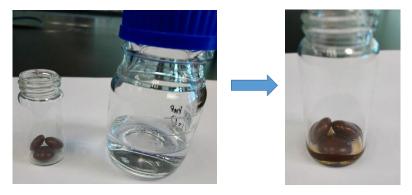
- 1 x Pencil
- 1 x Ruler
- 1 x Paper clip
- 2 x 20-mL vial
- 3 x Droppers
- 2 x Micro-centrifuge tube
- 1 x Chromatography paper (8.5 cm x 5 cm)
- 1 x Chromatography chamber (250-mL beaker)
- 1 x Watch glass
- 1 x A small magnet
- 5 x Pipette tips (for applying samples or standards)
- 1 x Hair dryer
- 1 x Timer

## Reagents and chemicals

- Isopropyl Alcohol
- 5 % Ammonia Solution
- Sunset Yellow (E110)
- Allura Red (E129)
- Brilliant Blue (E133)

## Experimental procedures

- A. Extraction of colorants from food (e.g., candies)
  - 1. Place three identical candies into the vial and add 1 mL of extraction solvent.

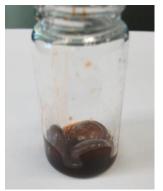


2. Screw the cap and shake until almost all the covering food colorants are dissolved. Then discard the candies carefully.





3. Add three more identical candies in the same vial and repeat step 2.





4. Use a dropper to transfer all extract from the vial into a micro-centrifuge tube.



- 5. Put the micro-centrifuge tubes into a centrifuge and spin at 13,000 rpm for 3 minutes.
- 6. After centrifugation, a clear extracted sample is obtained.



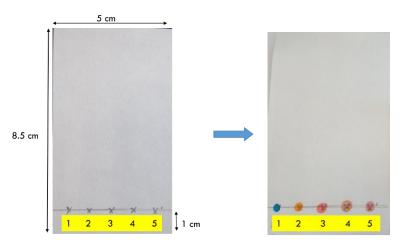
Undissolved solid would settle at the bottom

(left: before centrifugation; right: after centrifugation)

- B. Analysis of colorants using paper chromatography
  - 1. Prepare a strip of chromatography paper with dimension of 5 cm × 8.5 cm.
  - 2. Use a pencil to draw a horizontal line at 1 cm from the shorter edge of the strip and mark five crosses (three standards and two samples).
  - 3. Use pipette tips to spot the chemical standards and sample solutions on the paper strip.

[Tips: 1 to 2 spots are normally good enough for colorants with satisfactory intensity. Otherwise, repeated spotting is required.]

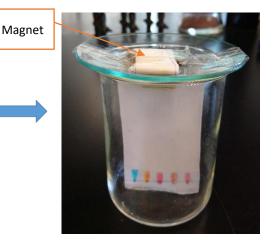
4. Leave the paper strip stand 2 minutes for air dry.



(1: Brilliant Blue, 2: Sunset Yellow; 3: Allura Red; 4: Sample A; 5: Sample B)

- 5. Add 7 mL developing solvent into a 250-mL beaker.
- 6. Clip the paper strip with a binder clip.
- 7. Attach binder clip to the convex side of a watch glass with a magnet.
- 8. To lay down the strip very slowly and carefully into the beaker and make sure the colored spots entirely above the surface level of the developing solvent.





- 9. Allow the solvent to move up and develop for 15 minutes.
- 10. Take out the strip and mark the solvent front.
- 11. Dry the strip with a hair dryer (or by air dry).
- 12. Use a pencil to mark down the position of the colorants on the strip and find out the distance each spot travelled (Figure 3).

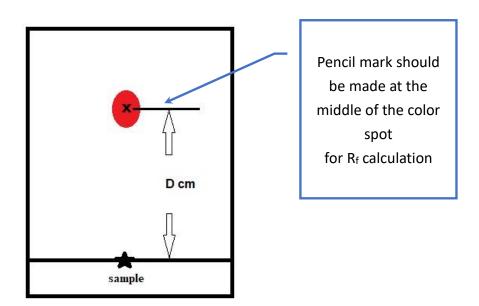


Figure 3: Example of measurement of the spot travelled.

- 13. Compare the final positions of the colorants in the sample.
- 14. [Challenge] Calculate the R<sub>f</sub> values of the colorants in the sample.

## Safety precaution

- Observe the standard safety procedures for laboratory activity;
- Put on the safety goggles, laboratory coats, and gloves;
- Material Safety Data Sheet (MSDS) of the chemicals are available online on the website of MSDSonline.com

## **Data Sheet**

	Sample A	Sample B
Color of Sample		
Number of Candies Used		
Volume of Extraction		
Solvent Used (mL)		
Color of the Extracted		
Sample Solution		

	Standard 1	Standard 2	Standard 3
Name of Colorant			
E Number			
Color of the Colorant			

#### Distance of Solvent Front = \_\_\_\_\_ cm

	Number of Spots	Distance of Travel	R <sub>f</sub> Value
	Developed	(cm)	Rt Value
Standard 1			
Standard 2			
Standard 3			
Sample A			
Sample B			

Name of colorant(s)

Sample A contains: \_\_\_\_\_

Sample B contains: \_\_\_\_\_

## Questions

1. Why is a pencil used to mark on the chromatography paper, instead of using a ball pen or a marker pen?

2. Why is centrifugation needed to pretreat the sample solution?

3. Explain how you identify different components of your unknown mixture(s) in this experiment.

## **Extended Reading**

The *Coloring Matter in Food Regulations* (Cap. 132H) stipulate the permitted coloring matter that can be used in food in Hong Kong. No coloring matter is permitted to be added to meat, fruit, or vegetable that are in a raw and unprocessed states. According to Centre for Food Safety of the Government, nowadays, there are more than 50 types of coloring matters allowed for food uses (e.g., sunset yellow). Starting from July 2007, the use of food additives, including coloring matter in pre-packaged food, must be listed by their functional classes and specific names or identification numbers. For example, if sunset yellow is used, it would be labelled on the packing as "Sunset yellow" or named as "E110". **E numbers** ("E" stands for "Europe") are codes for substances as food additives used within the European Union.

Since the personal-care products such as shampoo and mouth wash mainly apply on skin and hair, a wide range of colorants could be used. In Hong Kong, personal-care products are defined as consumer goods, which are subject to the regulation of the *Consumer Goods Safety Ordinance* (Cap. 456). The information of colorants used should be listed in the ingredient part on the package of product with prefix "CI" (<u>C</u>olor <u>I</u>ndex No.) (e.g., CI 42053 for Fast Green).

## Module 2 – Screening Test for Plastic Type of Plastic Products

#### Introduction

Plastics are a wide range of synthetic or semi-synthetic materials that use polymers as a main ingredient. Polymers are often made of carbon and hydrogen, and sometimes oxygen, nitrogen, sulphur, chlorine, fluorine, phosphorous, or silicon. Their elasticity makes it possible for plastics to be molded, extruded, or pressed into solid objects of various shapes. This adaptability, plus a wide range of other properties, such as being light weight, low electrical conductivity, durable, and inexpensive to produce, allows plastics to be made into great variety of products.

Most plastics are not biodegradable. Instead, plastics break into smaller and smaller fragments known as microplastics. They are much more difficult to be removed from the ocean and end up being swallowed by fish and other marine animals, as well as birds. Degraded plastic waste can directly affect humans via both direct consumption (by drinking water) and indirect consumption (by eating animals), harming our health.

Approximately 10,000 tons of municipal solid wastes are disposed into our landfills daily. More than 20% of them is plastic waste, weighing as much as 90 double-decker buses. To reduce the amount of waste generated and to improve overall waste management processes and programmes, three R's: Reduce, Reuse, and Recycle should be put into practice.



Recycling is the process to convert waste materials into new materials and objects. As plastic waste is non-biodegradable and will last for many years which cause a lot of serious environmental pollution problems, recycling the plastic waste had become the most important job. In order to facilitate the process of plastic recycling and further upcycling, it is necessary to identify the plastic type because some of the plastics are not recyclable. Moreover, the upcycling process requires the plastic to be of the same type, which it is needed to separate/isolate different kinds of plastic from a mixture.

All plastic products are often stamped with a resin code, which is a number between 1 to 7 in a small arrowed triangle. This number represents the kind of plastic resin of the product is made from in order to facilitate an easier recycling or other reprocessing.

#### Principles of the experiment

The density of a substance is its mass per unit volume. For example, 1 mL of pure water weighs 1 gram, so the density of pure water is 1 gram per 1 mL (or 1 g/mL).

In this experiment, a mixture containing three common types of plastics (Table 1) will be separated by the physical method based on the density. The mixture is put into water. Polypropylene (PP) with lower density than water will float while Polystyrene (PS) and Polyvinyl chloride (PVC) with higher density will sink. By repeating this process with a salt solution (density 1.1 - 1.2 g/mL), PS will float while PVC will sink. The principle is applied to a mixture of weighed plastic standards. It is expected that each fraction should finally contain one type of plastic (proved by the weights), so tentative identification is also achieved (Figure 1). Finally, the method is applied to separate a plastics sample and find out the percentage of each plastic in the sample.

Plastics	Density (g/mL)
Polypropylene (PP)	0.90 - 0.91
Polystyrene (PS)	1.05 - 1.07
Polyvinyl chloride (PVC)	1.20 - 1.30

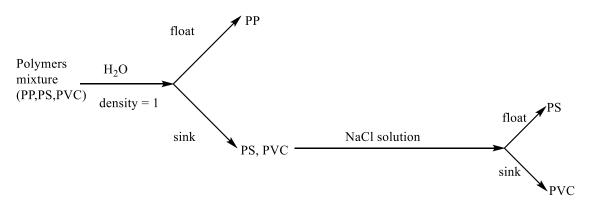


Figure 1. Scheme for separation/isolation and tentative identification of the plastic.

## **Intended Learning Outcomes**

After the activity, the student is expected to be able to:

- 1. understand the importance of environmental testing services and the role played by testing in daily life;
- 2. set up a screening test for plastic based on solutions of different densities;
- 3. identify the plastic type of different plastic products by flotation method;
- 4. acquire the basic concepts of STEM.

## Experiment

Apparatus

- 3 x 50 mL beakers
- 2 x 1 L beakers
- 1 x 100 mL measuring cylinder
- 6 x Watch glasses
- 1 x Glass rod
- 1 x Spatula
- 1 x Sieve
- 1 x Table spoon
- 1 x Analytical balance
- 1 x Oven

## Reagents and chemicals

- Polypropylene (PP)
- Polystyrene (PS)
- Polyvinyl chloride (PVC)
- Sodium chloride
- DI water



Figure 2. Polypropylene.



Figure 3. Polystyrene.



Figure 4. Polyvinyl chloride.

## Experimental procedures

- A. Preparation of sodium chloride solution
  - Dissolve 115 g sodium chloride in 800 mL DI water (The density of this solution should be 1.1 – 1.2 g/mL.)
- B. Separation of plastic standards
  - 1. Weigh approximately 2 g of polypropylene (PP), 3 g of polystyrene (PS), and 4 g of polyvinyl chloride (PVC) standard and put them into a 1 L beaker.
  - 2. Add 800 mL DI water into the beaker.
  - 3. Stir for a few minutes.
  - 4. Collect the floating plastics using a sieve and place them onto a watch glass which should be the PP standard.

- 5. Discard the DI water carefully.
- 6. Transfer the sunken plastics to another beaker containing 800 mL of sodium chloride solution.
- 7. Stir for a few minutes.
- 8. Collect the floating plastics using a sieve and wash them with tap water, and then, place them onto a watch glass which should be the PS standard.
- 9. Collect and wash the sunken plastics with tap water and place them onto a watch glass which should be the PVC standard.
- 10. Put the three watch glasses with different plastic standards into a 50 °C oven for 30 minutes.
- 11. After 30 minutes, take out the watch glasses and let them cool down to room temperature.
- 12. Weigh each portion of plastics collected and compare the results with the weight of plastic standards mixed at the beginning.
- C. Separation of plastic products
  - 1. Weigh 10 g plastic products and put them into a 1 L beaker.
  - 2. Repeat the above steps B2 B11.
  - 3. Weigh each portion of plastics collected and identify the type of plastic with respect to the results in Part B and report the percentage of them in sample.

## Safety precaution

- Observe the standard safety procedures for laboratory activity;
- Put on the safety goggles, laboratory coats, and gloves;
- Material Safety Data Sheet (MSDS) of the chemicals are available online on the website of MSDSonline.com.

## **Data Sheet**

#### **Observation**

	Color
Sodium chloride	
Sodium chloride solution	
Polypropylene standard	
Polystyrene standard	
Polyvinyl chloride standard	

#### Separation of plastic standards

	Туре	Weight mixed	Weight after separation
Plastic floats on DI water			
Plastic floats on salt water			
Plastic sinks in both liquid			

#### Separation of plastic products

Weight of plastic products: \_\_\_\_\_ g

	Туре	Weight	Percentage by weight
Plastic floats on DI water			
Plastic floats on salt water			
Plastic sinks in both liquid			

# Questions

1. What is density?

2. Give some examples of plastics with chemical names or abbreviations.

3. What is the nature of plastic?

4. Are all kinds of plastic bio-degradable?

5. Why are most kinds of plastic put together while they need to be separated for the recycling process?

6. How to separate plastics in the brown recycling bin with some metal containers?

## Module 3 - Analysis of Bacteria in Environmental Samples

#### Introduction

Microorganisms (or microbes) are present almost everywhere, but they are usually too tiny to be seen by the naked eye. They may include bacteria, fungi, algae, and viruses. Though microorganisms play an important role in maintaining the relationships among all life forms on Earth, some microorganisms can cause disease. Thus, we often try to reduce harmful microorganisms to acceptable levels with proper hygiene techniques. In this experiment, we will (Activity 1) reveal the existence of microorganisms from our surrounding surfaces, and (Activity 2) test the effectiveness of sanitizing products to a common type of microorganism.

#### **Activity 1. Surface studies**

We cannot see microorganisms with the naked eye, but when we collect them from our local environment and transfer them onto a solid nutrient surface (nutrient agar), they will grow, form groups, and become big enough to be visible "spots" (colonies). To transfer the sample to the nutrient surface, we will use the streak plate method to spread the microbes across the plate. After that, we incubate the agar plate to allow the bacteria to grow. We then apply the plate count method (by counting the number of colonies) to estimate the number of microbes collected form the tested surfaces. It is assumed that each colony is amplified from a single viable microbial cell, so this helps us "see" the microbes from our local environment. By counting the numbers of colonies for different plate samples, we can easily compare the cleanliness of surrounding surfaces. Surface studies is widely used in the food industry to maintain good food hygiene.

#### Activity 2. Performance check of sanitizing products

Next, we will try to see how effective the common sanitizing products are in reducing the number/growth of *S. epidermidis* using an adapted Kirby-Bauer disk diffusion test. *S. epidermidis* is a microorganism often found on our skin and normally does not cause disease on healthy people, so it is relatively safe and generally considered suitable for school laboratory classes under proper supervision. In the adapted Kirby-Bauer disk diffusion test, we will first prepare a confluent lawn of S. epidermidis on nutrient agar plate by the spread plate method, so that the bacteria will grow uniformly on the agar surface. Then we will soak different filter paper with different sanitizing solutions and put them on the agar. If the tested solution is able to inhibit (stop) bacterial growth, no bacteria will grow around the filter paper and a clear circular zone called "zone of inhibition" will be observed. The size or diameter of these zones of inhibition is indicative

of the strength of the applied sanitizing solution. This method is commonly used in antibiotic susceptibility test in clinical laboratories.

## **Intended Learning Outcomes**

After the activity, the student is expected to be able to:

- 1. understand the importance of testing services in assuring the quality and safety of sanitizing products, and the role played by testing in daily life;
- 2. analyze the amount of bacteria in environmental samples by plating method;
- 3. evaluate the effectiveness of different sanitizing products;
- 4. acquire the basic concepts of STEM.

## Experiment

Apparatus

- 2 x 250 mL reagent bottles
- 1 x Autoclave
- 1 x Water bath or oven (at 60°C)
- 8 x 100-mm sterile, disposable Petri dishes
- 1 x Bunsen burner (optional)
- 1 x Big plastic bag (optional, for agar plates storage)
- 6 x Resealable bags, 6 cm x 9 cm (for cotton swabs)
- 6 x Sterile cotton swabs
- 1 x Permanent marker
- 1 x Tube rack (for 1.5 mL centrifuge tubes)
- 6 x 1.5 mL sterile centrifuge tubes
- 1 x Sterile bacterial culture tube
- 1 x 37°C incubator with shaking function
- 1 x 1 mL pipette
- 1 x 200 µL pipette
- 1 x Sterile 1 mL pipette tips, racked
- 1 x Sterile 200 µL pipette tips, racked
- 1 x Sterile spreader
- 1 x A sterile petri dish containing 8 pieces of sterile filter paper (6 mm diameter)
- 1 x Forceps
- Some paper towels

## Reagents and chemicals

- Nutrient agar, dehydrated
- Nutrient broth, dehydrated

- DI water
- Sterile DI water
- 75% ethanol
- Household bleach solution
- Dettol solution
- S. epidermidis

## Experimental procedures

(To avoid contamination, DO NOT touch the materials on the lab bench barehanded and DO NOT remove the covers or caps until the moment you are going to use the materials)

A. Surface studies

Environmental sampling:

- 1. Each group is provided with six sterile cotton swabs that are individually packed in resealable plastic bags.
- 2. Choose five different surfaces you are interested in testing, and perform the sampling accordingly:
  - 2.1. Take out the cotton swab carefully by holding one end. Wet the other end by dipping it in the sterile deionized water provided, and roll the wet swab over the surface back and forth for a few times.
  - 2.2. Put the swab back to the resealable bag, seal the bag, and label the bag with sample name.
  - \* Remember to bring a marker or a pen along for labeling.
  - \* Examples of surfaces: coins, phones, keyboards, computer mice, refrigerators, water faucets, press buttons of water dispensers, toilet flushing buttons, stationery, staircase handrails, vending machines, facilities in lecture rooms or common rooms, shoes, skin, etc.
  - \* Avoid any dangerous places and dining area.
- 3. You should always prepare a control sample by dipping cotton swab with sterile water only.

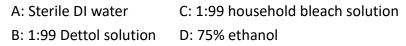
Inoculation using streak plate method:

- 4. Apply aseptic techniques.
  - (i.e., free from contamination of microorganisms)
  - 4.1. Wipe your gloves and the work area with 75% ethanol.
  - 4.2. Wipe the surface of the marker pen with 75% ethanol.
  - 4.3. Avoid touching agar surface with your hands.

- 5. Each group needs six agar plates in this part. Label around the edge of the bottom (agar side) of the agar plates with group number, sample name and the date.
- Inoculate samples from each cotton swab to corresponding plate, using the streak plate method. There should be six samples in total: one control and five target samples. For each sample:
  - 6.1. Take the cotton swab out of the bag carefully.[Note: Avoid touching the end of the swab exposed to samples.]
  - 6.2. Lift the lid of corresponding agar plate just enough to insert the swab, drag the swab over the entire surface of the agar back and forth in a zigzag motion. Close the lid as soon as you are finished.

Incubation:

- 7. Incubate the agar plates upside down (label side up) at 37°C for 2 days.
- B. Performance check of sanitizing products by adapted Kirby-Bauer disk diffusion test
  - 1. Each group is provided with a set of sterile DI water, 75% ethanol, 1:99 household bleach solution, 1:99 Dettol solution and diluted *S. epidermidis* culture, and a plate with 8 pieces of sterile filter paper (6 mm diameter) to each group.
  - 2. Two agar plates are required in this part (two replicates). Label the plates as image below.



- 3. You are required to grow *S. epidermidis* using spread plate method.
  - 3.1. Apply aseptic techniques.
    - 3.1.1. Wipe your gloves, work area, surface of the pipettes, tip racks, and packing of the spreader with 75% ethanol
  - 3.2. Pipette out 100  $\mu l$  diluted bacterial culture carefully and add onto each



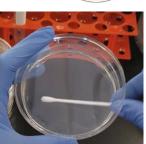
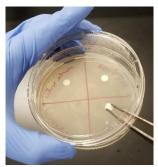






plate.

- 3.3. Spread the culture evenly over the agar surface using a sterile spreader by rotating the Petri dish underneath at the same time.
- 4. Introduce different sanitizing products to the bacteria.
  - 4.1. Hold a new sterile filter paper by a sterile forceps, and wet the filter paper in a tube with corresponding sanitizing solution.
  - 4.2. Lift the lid of agar plate a little bit; place the wet filter paper into corresponding quadrant, as shown in image on the right. Close the lid as soon as you are finished



- 5. Carefully place the agar plates face up in a 37°C incubator for 2 days.
- C. Colony counting and measure the zone of inhibition
  - 1. Two days later, you are required to screen the colony formation on the plates, count the number of colonies (DO NOT open the petri dish), and record their results.

[Note: It is assumed that each colony is amplified from a single cell of the microorganism. Whether a result with good colony separation can be obtained depends on the streaking plate technique and starting microbial density.]

2. Also, you are required to examine the zone of inhibition, and measure the diameter of the clear zones by holding a ruler over the back of the inverted plate (DO NOT open the petri dish).

[Note: The plates can be examined every day to make sure that the agar plates are not contaminated or that the filter paper remains in position.]

## Safety precaution

- Observe standard safety procedures for laboratory activity;
- Put on safety goggles, laboratory coats, and gloves;
- Material Safety Data Sheet (MSDS) of the chemicals are available online on MSDSonline.com;
- Wash hands thoroughly before leaving the laboratory.

## Data Sheet

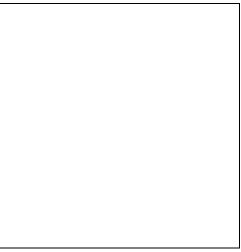
# A. Surface studies

Attack incorrection the faller	الالمعممة اممم مامامة ممتني	المعر معامية بالمعام مع	محمله مامحم
Attach images in the follow	wing table and record t	ne colony number i	or each plate.

Sample	Image	Colony number

## B. Performance of sanitization products

Attach a representative image below, and record the diameters of the clear zones.



	Zone of Inhibition (mm)			
	Plate 1 Plate 2 Mean			
(A) DI water				
(B) 1:99 Dettol				
(C) 1:99 bleach				
(D) 75% ethanol				

## Questions

1. According to your results, which surface was the dirtiest?

2. Among the three given sanitizing solutions, which one(s) is/are the strongest disinfectant(s) against *S. epidermidis?* 

3. What type(s) of microorganisms do you expect to grow on your agar plates?

4. Why do we need to apply aseptic techniques and use sterile items during some steps of the experiment?

5. Suggest two personal hygiene measures to prevent infectious disease.

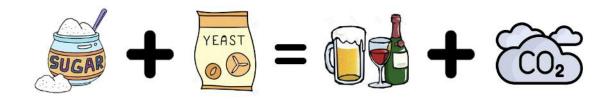
## Module 4 – Wine-Making and Analysis of Alcohol in Beverage

#### Introduction

#### A. Fermentation

Yeast is a single-celled living thing and is found everywhere in the world around us. Yeast gets energy from food in the form of sugar. This can be pure sugar, honey, molasses, maple syrup, or fruit juices containing natural sugars

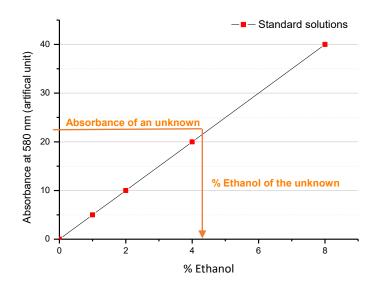
Yeast has been used for thousands of years to make alcoholic beverages, such as wines and beers. In the absence of oxygen, most of the sugar is converted to carbon dioxide and ethanol by yeast. This process is known as fermentation. Ethanol produced by fermentation of sugars is the basis of the wine and beer industry.



#### B. Analysis of ethanol based on dichromate ions

The ethanol content in common beverages (i.e., wine, beer, Chinese wine, etc.) can be quantitatively analyzed by the reaction between ethanol ( $CH_3CH_2OH$ ) and dichromate ions ( $Cr_2O_7^{2-}$ ). The dichromate ions serve as a color indicator that there will be a color change from yellow orange to blue green upon reacting with ethanol. The extent of color change can be monitored by measuring the absorbance of yellow light (580 nm) with a simple colorimeter.

To determine the percentage of ethanol in an unknown sample, a set of 0 - 8% ethanol standard solutions and their corresponding absorbance at 580 nm are measured for establishing a calibration curve. Since the absorbance at 580 nm is proportional to the % ethanol, the calibration curve should show a linear relationship (as shown in the figure below). Then, the % ethanol in an unknown sample can be estimated by fitting its absorbance into the calibration curve. This calibration curve method is commonly used in analytical science.



[Note: According to the laws of Hong Kong, sale and supply of intoxicating liquor to persons under the age of 18 ("minors") in the course of business, through face-to-face or remote distribution, or by vending machines is prohibited.]

## **Intended Learning Outcomes**

After the activity, the student is expected to be able to:

- 1. understand the importance of testing services in assuring the quality and safety of food and the role played by testing in daily life;
- 2. produce a wine from the respective raw materials under fermentation;
- 3. analyze the alcohol content in beverage by colorimetry;
- 4. acquire the basic concepts of STEM.

#### Experiment

Apparatus

- 1 x 500 mL two necked round bottom flask
- 1 x Glass stopper
- 1 x 15 cm long rubber tubing
- 1 x Dropper
- 1 x Stirrer
- 1 x 500 mL measuring cylinder
- 1 x Screw adapter
- 1 x 10 mL test tube
- 2 x Clamps
- 8 x Test tubes (can be replaced by cuvettes)
- 1 x Test tube rack (3D-printing rack can be designed as cuvette holder)



Figure 1. Glass apparatus

- 5 x 100 mL volumetric flasks
- $1 \times 10 200 \mu L$  autopipette
- 1 x 1 5 mL autopipette
- Pipette tips for autopipette
- 1 x Heater
- 1 x 400 mL beaker
- 1x Mobile device (iOS or Android)

## Reagents and chemicals

- Glucose
- Yeast (wine production)
- Yeast
- Ethanol
- Potassium dichromate
- Sulphuric acid

## Experimental procedures

- A. Wine-making using fermentation
  - 1. Add 8 g glucose, 0.2 g yeast extract, 2 g yeast compound (wine yeast), and 400 mL DI water into a 500 mL round bottom flask.
  - 2. Stir the mixture gently.
  - 3. Assemble the setup as below.



- 4. Incubate the mixture at room temperature for 2 hours.
- B. Analysis of alcohol in beverage using ChemEye (smartphone colorimetry) Ethanol calibration standards:

	0% (Blank)	1%	2%	4%	8%
Ethanol solution	200 μL	200 μL	200 μL	200 μL	200 μL
Potassium dichromate solution	2.0 mL	2.0 mL	2.0 mL	2.0 mL	2.0 mL

1. Prepare a set of ethanol calibration standards in test tubes.

- 2. Heat the test tubes in hot water bath for 5 minutes, then cool down to room temperature.
- 3. Add 5.0 mL DI water into each test tube.
- Wine sample:
- 4. Mix 200  $\mu$ L of sample solution with 2.0 mL potassium dichromate solution in a test tube.
- 5. Heat the test tube in hot water bath for 5 minutes, and then cool down to room temperature.
- 6. Add 5.0 mL DI water into the test tube.

Measurement using ChemEye:

7. Install "ChemEye" to the mobile device with the QR codes provided.





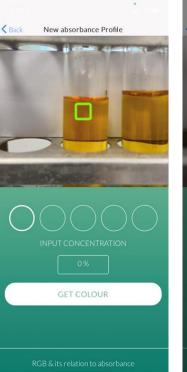
For Android device

8. Place the test tubes in front of white background.



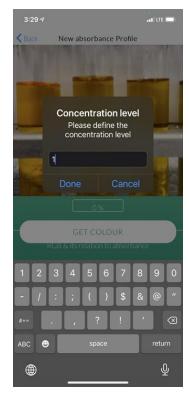
9. Follow the procedures to create the calibration curve and detect the sample:

3:27 -7	Back Choose detection method	Back Absorbance Profile
ChemEye Dept. of Chemistry Hong Kong Baptist University START	ABSORBANCE EMITTANCE	
FAQ FAQ 1. Open the App and press "START"	What is absorbance and emmitance? 2. Choose "ABSORBANCE"	NEW PROFILE What is absorbance profile? 3. Click on "NEW PROFILE"
Add Absorbance Profile	Input Concentration	Back Input Data Points
INPUT PROFILE NAME	INPUT CONCENTRATION UNIT	INPUT NUMBER OF COLOR POINTS
What is absorbance profile?         "test"       testing       tests         q       w       e       r       t       y       u       i       o       p         a       s       d       f       g       h       j       k       i	% COMMON UNITS: ppm pob ug/mL ng/mL mM uM % NEXT	S NEXT
"test" testing tests qwertyuiop asdfghjkl ☆ zxcvbnm ⊗	COMMON UNITS: ppm ppb up/mL ng/mL mM u/M %	



7. Place the green square on the standard solution and press "GET COLOR"

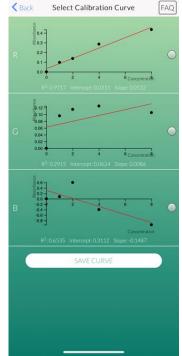




8. RGB value obtained

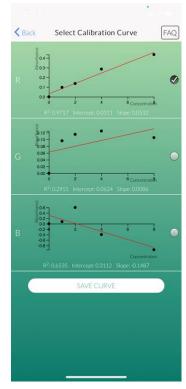


10. Repeat all standard solutions. Click on "FINISH"



11. Red, green, and blue color calibration curves obtained.

9. Enter the respective concentration



12. Red curve selected in this experiment.

#### 30

		1			i ne est		a se an
Back	Detect Concentration	-	Back	Compare Blanks	FAQ	Back	Detect Concentration
Name: y=0.0	ALCOHOL TEST (Chann 491x+-0.0381 (R <sup>2</sup> =0.93	el R) 91)		2: ALCOHOL TEST (Chanr 0491x+-0.0381 (R <sup>2</sup> =0.9)		Nam y=(	e: ALCOHOL TEST (Channel R) .0491x+-0.0381 (R <sup>2</sup> =0.9391) R: 147 G: 85 B: 0
	GET COLOUR		ST		: 0		
	DONE			GET COLOUR			GET COLOUR
				DETECT			DONE
	e best straigh on with R <sup>2</sup> sl		14. "D	ETECT" the s	ample		15. Detected centration shown

# Safety precaution

- Observe the standard safety procedures for laboratory activity; •
- Put on the safety goggles, laboratory coats, and gloves;
- Material Safety Data Sheet (MSDS) of the chemicals are available online on the website of MSDSonline.com.

## **Data Sheet**

Qualitative analysis by ChemEye

Sample	Description	Labeled	Ethanol	R,G,B
		content	detection result	Line
		(%)	by ChemEye (%)	selection
1.				
2.				
3.				

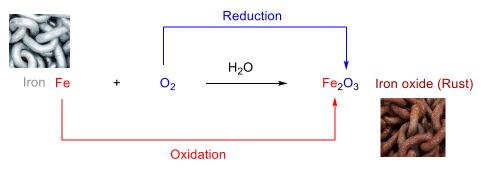
## Questions

1.	What is the role of yeast in fermentation?
2.	What conditions may affect the fermentation reaction?
3.	Give examples of redox reactions in daily life. (referred to the extended reading)

# Extended Reading

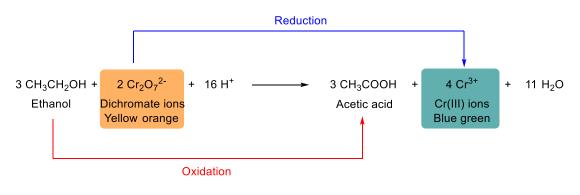
## **Redox Reaction**

An oxidation-reduction reaction (also known as a redox reaction) is a type of chemical reaction that involves a transfer of oxygen. For example, iron rusting is a redox reaction in which iron (Fe) is oxidized to iron oxide (Fe<sub>2</sub>O<sub>3</sub>) by oxygen in the presence of water.



In this experiment, the reaction between ethanol ( $CH_3CH_2OH$ ) and dichromate ions ( $Cr_2O_7^{2-}$ ) is also a redox reaction. As shown below, ethanol in the alcoholic beverages will be oxidized to acetic acid ( $CH_3COOH$ ) by a dichromate solution (a yellow orange color solution) under an acidic condition. Meanwhile dichromate will be reduced to Cr(III) ions ( $Cr^{3+}$ ), which is in blue green color. The amount of Cr(III) ions generated can be

determined by measuring the absorbance of yellow light (580 nm) with a simple colorimeter.



## Module 5 – Slime-Making and Analysis of Borax in Slime

#### Introduction

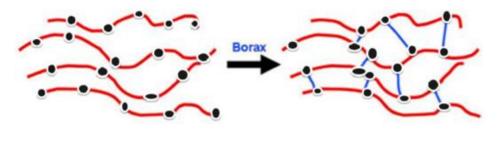
Playing slime is a very common experience for kids and most of them love it. Making slime is also extremely fascinating, which the starting materials can be simply glue and water. With the addition of borax and well-mixing, a sticky mass, as known as slime, is formed. However, the safety of slime is always a concern since borax may cause some health risks, such as skin, eye, and respiratory irritation. This experiment will give you an opportunity to make slime and check the existence of borax.



#### Principle of the experiment

Slime consists of long molecules called polymers that move slowly against each other. The term, polymer, is a composite of the Greek words *poly* and *meros*, meaning "many parts". We encounter polymers every day, such as cellulose in plants, keratin in hair and nails, and all types of plastics in consumer products. PVA glue is also an example of polymer, composed of long chains of polyvinyl acetate.

Borax –  $Na_2[B_4O_5(OH)_4]\cdot 8H_2O$ ; sodium tetraborate – is a powdery white mineral, which has been used as a cleaning product for decades. When 2% borax solution is added to the glue solution (a mixture of water and glue in 1:1 ratio), the borate ions help to link the glue polymer chains together that they cannot move and flow easily. This structure is sticky which can be stretched, pulled, beaten and shaped, and is known as slime.





Slime

To detect borax in slime, turmeric powder can be applied as a screening test. Curcumin is a yellow natural pigment found in the turmeric powder. Curcumin reacts with borax to form a reddish compound (rosocyanine) and such color can easily be visualized. The reaction is highly sensitive that trace amount of borax can be detected.



To further determine the amount of borax in slime, acid-base titration is applied. HCl will first be added in excess to neutralize the borate ions to destroy the linkages among polymer chains. Upon the complete reaction with all the borate ions in the slime, the remaining HCl will then be determined by titration with NaOH using methyl red as the indicator (from red to yellow). Finally, the amount of borax can be calculated.

#### **Intended Learning Outcomes**

After the activity, the student is expected to be able to:

- 1. understand the importance of testing services in assuring the quality and safety of toy and the role played by testing in daily life;
- 2. produce a slime by mixing the respective raw materials;
- 3. analyze the borax content in slime by titration;
- 4. acquire the basic concepts of STEM.

# Experiment

Apparatus

- 1 x 50 mL burette
- 1 x Burette clamp
- 1 x Stand
- 1 x 15 mL pipette
- 1 x 100 mL measuring cylinder
- 4 x 150 mL beakers
- 2 x 100 mL volumetric flasks
- 1 x 1 L volumetric flask
- 1 x Spot plate

- 1 x Ziploc bag
- 2 x Stirring rods
- 5 x Droppers
- 1 x Disposable plastic cup

## Reagents and chemicals

- Ethanol
- Hydrochloric acid (HCl)
- Methyl red indicator
- Sodium hydroxide (NaOH)
- Borax
- White Glue
- Turmeric powder

## Experimental procedures

- A. Slime-making
  - 1. Add 50 mL of white glue and 50 mL of water in a disposable plastic cup and stir thoroughly to mix well. [Tips: You might use more or less amount of glue, as long as you maintain a 1:1 ratio between the glue and water.]
  - 2. [Optional] Add a few drops of food colorant and stir thoroughly to mix well.
  - 3. Add a few drops of borax solution at a time using a dropper to the glue-water mixture and stir thoroughly with stirring rod.
  - 4. Continue adding the borax solution until most of the glue-water mixture has turned into slime.

[Tips: DO NOT add too much borax solution. Stop adding the borax solution when there is still a little glue-water mixture left in the bottom of the cup.]

- 5. Remove the slime from the stirring rod with your fingers and work with your hands until it is no longer sticky. Store it in a Ziploc bag.
- B. Analysis of Borax in slime with curcumin
  - 1. Take a clean spot plate and mark them A, B, C.
  - 2. Add a few drops of glue solution into well A in your spot plate control.
  - 3. Put a few pieces of slime into well B and add several drops of hot water. Use a clean toothpick to mix the solution.
  - 4. Add a few drops of 2% borax solution into well C.
  - 5. Add 2 drops of turmeric indicator solution into wells A, B, C, respectively.
  - 6. Compare the color of the solutions to the control.

- C. Extension/Optional Learning Activity Analysis of Borax in slime using titration
  - 1. Weigh accurately 50 g of slime into a 150 mL beaker.
  - 2. Add 15.0 mL of 0.1 M HCl into the same beaker using pipette to dissolve the slime thoroughly using a stirring rod.
  - 3. Add 10 drops of methyl red indicator to the slime-HCl solution, i.e., red color.
  - 4. Titrate the resulting red solution with 0.1 M NaOH.

[Tips: Mix the solution well using a glass rod after each addition.]

- 5. The end point is reached when the color changes to light yellow.
- 6. Record the volume of sodium hydroxide solution used (mL) and calculate the borax content (%) in the slime.

$$Borax = \frac{(15 - volume \ of \ NaOH \ used) \times 0.019}{mass \ of \ slime \ used} \times 100\%$$

## Safety precaution

- Observe the standard safety procedures for laboratory activity;
- Put on the safety goggles, laboratory coats, and gloves;
- Material Safety Data Sheet (MSDS) of the chemicals are available online on the website of MSDSonline.com.

# Data Sheet

## Observation

- Slime-marking
- Analysis of Borax in slime with curcumin

	Well A	Well B	Well C
Color			

## • Analysis of Borax in slime using titration

Record the volume of NaOH used (mL) and calculate the borax content (%)

Mass of slime (g)	
Volume of HCl added (mL)	
Initial volume (mL)	
Final volume (mL)	
Volume of NaOH used (mL)	
Borax content (%) in the slime	

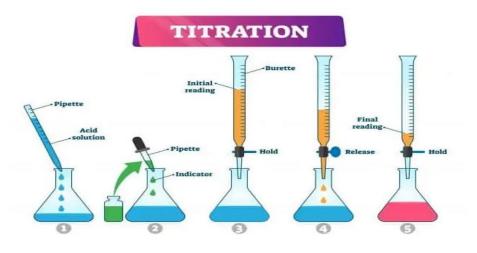
#### Questions

- 1. Is slime a liquid or solid? Give your reasons below.
- 2. Describe the properties of slime and how it feels in your hands.
- 3. What are some of the polymers that you encounter every day?

## **Extended Reading**

#### What is acid-base titration?

An acid-base titration involves a chemical reaction between an acid and a base, e.g., HCl and NaOH, respectively. The products of such a reaction are a salt (e.g., NaCl) and water. Since these solutions are colorless, it requires a color indicator to show the point at which the reaction is complete by changing the color. This indicator is a special type of substance that tells us whether the solution is more acidic or basic.



## Module 6 - Solar Cell Fabrication and Testing on its Electrical Properties

#### Introduction

Nowadays, non-renewable fossil fuels, such as oil, gas, and coal, are heavily used to produce electricity worldwide, so that scientists have been searching for alternative renewable energy sources that are pollution-free and cheap but can still provide high efficiency. As a result, there are tremendous research activities on the development of stable and high-efficiency solar cells that can directly convert solar energy into electrical energy in recent years.

A dye-sensitized solar cell is a low-cost solar cell belonging to the group of thin film solar cells. It is based on a semiconductor formed between a photo-sensitized anode and an electrolyte. Dye-sensitized solar cells (DSSC) have attracted much attention recently because of their good performance in converting solar energy into electricity using thin-films of organic and inorganic materials. They are also flexible, low in production cost, and easy to be fabricated.

DSSC is a technology that can be used to produce electricity in a wide range of light conditions including indoors and outdoors, enabling the user to convert both artificial and natural light into electrical energy to power a broad range of electronic devices.

#### Principle of a Dye-Sensitized Solar Cell

In this experiment, hands-on experiment on constructing a dye-sensitized solar cell (DSSC) using the natural dye from blueberries as a photosensitizer will be carried out. The schematic diagram showing the basic principle of a DSSC is shown in Figure 1.

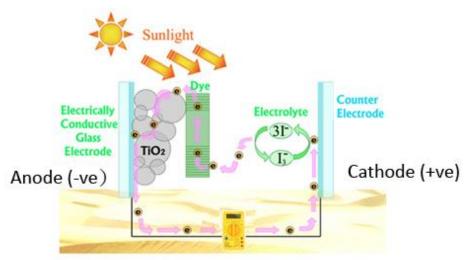


Figure 1. A schematic diagram of a typical dye-sensitized solar cell.

Typically, a DSSC is composed of a light-absorbing dye (blueberries in this experiment), titanium dioxide nano-crystalline material, a transparent electrically conductive glass electrode, a counter electrode, and a liquid electrolyte, which can convert light energy (sunlight) into electrical energy (electricity) upon light illumination.

## **Intended Learning Outcomes**

After the activity, the student is expected to be able to:

- 1. understand the importance of testing services in assuring the quality and safety of different electrical products and the role played by testing in daily life;
- 2. produce a dye-sensitized solar cell using natural dye as photosensitizer;
- 3. analyze the electrical properties of solar cell using multimeter;
- 4. acquire the basic concepts of STEM.

#### Experiment

Apparatus

- 2 x FTO conductive glasses
- 1 x Multi-meter
- 1 x LED torch
- 1 x Pencil
- 1 x Tape
- 1 x Small paper
- 1 x Forceps
- 1 x White tile
- 1 x Hotplate
- 1 x Glass rod
- 2 x Clips
- 1 x Pipet filler
- 1 x 25 mL beaker
- 4 x Wires with alligator clips
- 1 x Stopwatch with alligator clip
- 1 x Watch glass

#### Reagents and chemicals

- Ethanol
- Potassium iodide
- Iodine
- Ethylene glycol
- DI water

## (frozen fruit bought from supermarket)

• TiO<sub>2</sub> paste

Blueberries

## Experimental procedures

- A. Fabrication of a DSSC and Analysis of respective electrical properties
  - Get the prepared piece of coated TiO<sub>2</sub> conductive glass ready. Add the natural dye solution (from blueberries) on the coated glass by dropper and keep for at least 20 minutes. The white TiO<sub>2</sub> will change color as the dye is absorbed and complexed with the Ti(IV).
  - 2. Rinse gently with water then with ethanol. Blow to dry the coated glass.
  - 3. Pick another piece of glass to prepare the counter electrode. Identify the conducting side of the conductive glass first by using the multi-meter.
  - 4. Wipe off any fingerprints or oils using wet tissue with ethanol and dry it.
  - 5. Paint the entire conductive surface with pencil. A layer of carbon is now covered on the glass surface. Rinse it with ethanol and dry it thoroughly.
  - 6. Assemble the two coated glass plates together with an offset so that uncoated glass surface extends beyond the sandwich. Do not rub or slide the glass plates. Clamp the plates tightly together with clips.
  - Measure and write down the V<sub>oc</sub> (open circuit voltage) and I<sub>sc</sub> (short circuit current) of the fabricated solar cell under room illumination and LED torch before adding electrolyte.
  - Add a few drops of a triiodide solution (KI<sub>3</sub> solution) to the edge of the plate. Capillary action will cause the KI<sub>3</sub> solution to fill up between the sandwiched plates.
  - Connect the sandwich to the multi-meter using an alligator clip to each plate.
     [Hint: Do not scratch the surface of conductive glasses.]



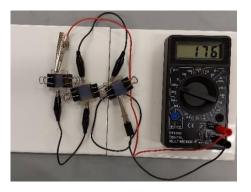




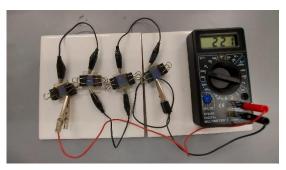
10. Measure and write down the  $V_{oc}$  (open circuit voltage) and  $I_{sc}$  (short circuit current) of the solar cell under room illumination, under LED torch, and in dark box.



- B. Power on the stopwatch by connecting several pieces of DSSCs in series
  - 1. Connect 3 pieces of DSSCs in series and measure the  $V_{oc}$  (open circuit voltage) and  $I_{sc}$  (short circuit current) of this system by multi-meter under illumination of LED torch.



2. Continue to add the solar cell (DSSC) to the system until the voltage is larger than 1200 mV, then disconnect the multi-meter.



3. Connect the system and check whether it can power on the stopwatch or not under the illumination of LED torch. Make down the number of pieces of solar cells are needed to switch on the stopwatch.



## Safety precaution

- Observe the standard safety procedures for laboratory activity;
- Put on the safety goggles, laboratory coats, and gloves;
- Material Safety Data Sheet (MSDS) of the chemicals are available online on the website of MSDSonline.com.

## Data Sheet

#### A. Fabrication of a DSSC and Analysis of respective electrical properties

Type of Berries used: \_\_\_\_\_

#### Before adding triiodide solution (electrolyte):

	V <sub>oc</sub> (open circuit voltage)	I <sub>sc</sub> (short circuit current)
Room Illumination	(mV)	(μA)
Under LED Torch	(mV)	(μΑ)

.:. Under no electrolyte conditions, the solar cell worked / did not work\*

#### After adding triiodide solution (electrolyte):

	V <sub>oc</sub> (open circuit voltage)	I <sub>sc</sub> (short circuit current)
Room Illumination	(mV)	(μΑ)
Under LED Torch	(mV)	(μΑ)
In the dark box	(mV)	(μΑ)

:. Increase light intensity: the voltage will be *increased* / *decreased* \*

#### B. Power on the stopwatch by connecting several pieces of DSSCs in series

#### **Under LED Illumination**

No. of solar cell	V <sub>oc</sub> (open circuit voltage)	I <sub>sc</sub> (short circuit current)
3	(mV)	(μA)
4	(mV)	(μA)
5	(mV)	(μA)

No. of solar cells required to power on the stopwatch\_\_\_\_\_

Estimate the minimum voltage that can power on the stopwatch\_\_\_\_\_mV

.: Several solar cells connected in series: the voltage will be increased / decreased\*

\*Circle the appropriate answer in each question

## Questions

1. How do the voltage and the current generated by the solar cell change when various intensity of light is shone on the solar cell?

2. What is the purpose of using ethanol to wash the dye-coated TiO<sub>2</sub> glass substrate?

3. What are the advantages of DSSCs for commercial applications?

4. The major disadvantage of the DSSCs is the use of the liquid electrolyte which has temperature stability problems. What is the worst situation that could happen at low temperatures and high temperatures?