

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

**Teacher's Guide**

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A Project Commissioned by  
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### Important Note

Teachers should ensure the safety of all experimental activities and must be thorough in preparation. Before each experiment, teachers must read the **safety precaution** in this Guide carefully, and give clear instructions to students and remind them of the potential hazards and safety precautions to take (especially on relation to the handling of chemicals). Personal protective equipment, eye wash unit and first-aid box should be ready. Teachers should give sufficient supervision and guidance to students during experiments, and maintain good control of class discipline. Advice and information offered in this Guide are by no means exhaustive and do not preclude the need for exercising care and good judgement at all times in safeguarding against accidents.<sup>1</sup>

<sup>1</sup> Adapted from "Safety in Science Laboratories" published by the Education Bureau in 2013. When in doubts, please also refer to this document (or its latest version) and the website of "Resources on Laboratory Safety and Management" at [http://cd1.edb.hkedcity.net/cd/science/laboratory/content\\_safety.html](http://cd1.edb.hkedcity.net/cd/science/laboratory/content_safety.html).

## **Knowing the Testing and Certification Sector in Hong Kong**

### **Hong Kong's Testing and Certification Sector**

The testing and certification sector plays a vital role in our daily life, helping assure the quality and safety of products. The Government Laboratory provides a wide range of analytical, investigatory, and advisory services and support to enable the Government to meet its responsibilities in public health and safety, environmental protection, law enforcement, etc. Private laboratories are playing an important role in the analysis of food, water, and environmental samples. Medical laboratories provide support to the medical sector in the diagnosis of illnesses. Construction materials laboratories and the corresponding inspection bodies contribute to ensure the safety of buildings and constructions.

The testing and certification sector also plays an important role in supporting the external trade of Hong Kong. It provides a high volume of testing and inspection services for consumer products manufactured in Hong Kong and the neighbouring regions. These products include toys, electrical and electronic goods, textiles and garments, and footwear. The sector also provides quality management system certification service (such as ISO 9001) for businesses. The sector is, therefore, an integral part of the overall manufacturing supply chain and contributing to Hong Kong's re-industrialisation. Through providing assurance to overseas buyers on the quality and safety of products, the sector is important to the economic development of Hong Kong.

During the COVID-19 pandemic, the testing and certification sector has vigorously assisted the community in fighting against the virus in different aspects. For example, the sector has served as the gatekeeper by ensuring the quality of a wide range of anti-epidemic items, such as surgical face mask, alcohol-based hand-rub, ultraviolet disinfection equipment, etc. Apart from the provision of COVID-19 nucleic acid and antibody testing services, the sector also took part in the sewage surveillance programme by testing for virus to identify high-risk locations.

### **Accreditation in Hong Kong**

Accreditation is the third-party recognition to affirm a testing and certification organisation's competence to carry out specific testing and certification services in accordance with international standards. Accreditation is open and voluntary in Hong Kong. It is currently provided by Hong Kong Accreditation Service (HKAS) under Innovation and Technology Commission. HKAS operates three accreditation schemes:

- (i) the Hong Kong Laboratory Accreditation Scheme (HOKLAS);
- (ii) the Hong Kong Certification Body Accreditation Scheme (HKCAS); and
- (iii) the Hong Kong Inspection Body Accreditation Scheme (HKIAS).

In order to get accredited status, a testing and certification organisation will need to be rigorously assessed by independent experts. After obtaining accreditation, the testing and certification organisation will still be subject to regular surveillance and re-assessment to ensure its continual conformity to the prescribed standards. Quality of test results provided by accredited testing and certification organisations is thus assured. For more details on the accreditation services of HKAS and the list of accredited establishments under HOKLAS, HKCAS and HKIAS, please visit HKAS's website ([www.hkas.gov.hk](http://www.hkas.gov.hk)).

### **Career in Testing and Certification Sector**

Given the growing emphasis on quality products and services nowadays, the demand for testing and certification services has been on a rising trend. The testing and certification sector has accorded great importance to talent development and is offering attractive career prospect.

Students with interest to join the testing and certification sector upon graduation are suggested to study at least one science subject, e.g. Biology, Chemistry, Physics, Combined Science, Integrated Science, etc. during their senior secondary years. They should also make an effort in building up their language skill and scientific thinking. At the post-secondary/university level, relevant disciplines include Science, Applied Science, Engineering, Fashion and Textiles, etc. A number of tertiary institutions in Hong Kong has also been offering academic programmes dedicated to Testing and Certification, ranging from the sub-degree to postgraduate levels. A list of the tertiary programmes can be found at the website of Hong Kong Council for Testing and Certification ([www.hkctc.gov.hk/en/career/edu/course\\_list.html](http://www.hkctc.gov.hk/en/career/edu/course_list.html)).

Teachers are encouraged to provide some background of testing and certification to students before starting the six laboratory activities. Students should gain some knowledge of the importance of testing and certification to their daily life, and the importance of the relevant sector to Hong Kong's economy and society. It may enhance student's interest by bringing science closer to them.

## Teacher's Guide

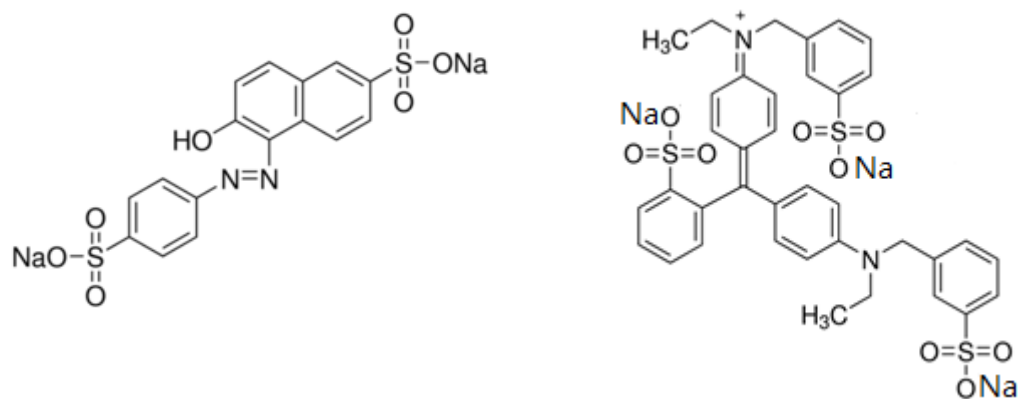
### Module 1 – Analysis of Colorants in Food and Personal-Care Products

#### Introduction

Colorants are widely used in food, cosmetic, and personal-care products. In general, the addition of colorants in food will make the products more attractive to consumer, while the use of coloring agents in personal-care products is mainly for decorative purpose [1].

The colorants, organic or inorganic compounds, are being added during the preparation of the consumer products [2]. They are either derived from natural sources or made synthetically. For the natural colorants, it is obtained from plants (e.g., carotenes from carrot) or minerals. However, they are normally unstable and easily degraded by light and temperature. Therefore, the application of synthetic colorants is more popular. This is because the synthetic colorants have a lot of advantages, such as high solubility in hydro- or oil-based consumer products; more resistant to light, oxygen, and pH change; long-lasting time; and low price. Nowadays, natural colorants have been partially replaced by synthetic colorants [3].

Synthetic colorants are divided into five classes: azo, triarylmethane, xanthene, indigo and quinolone classes. They usually exist as a sodium salt in order to increase the water solubility [4]. Tartrazine and Sunset yellow are typical examples of azo group dyes, while Brilliant blue belongs to the triarylmethane group (Figure. 1).



**Figure 1.** Chemical structures of Sunset yellow (left) and Brilliant blue (right).

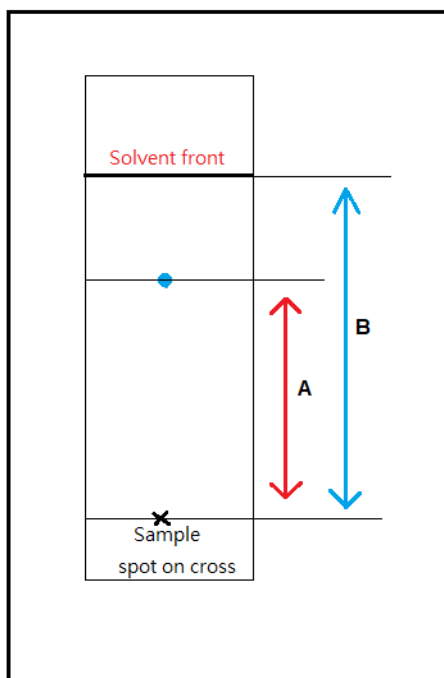
The *Coloring Matter in Food Regulations* (Cap. 132H) stipulate the permitted coloring matter that can be used in food in Hong Kong [5]. No coloring matter is permitted to be added to meat, fruit, or vegetable that are in a raw and unprocessed states. According to Centre for Food Safety of the Government [6], 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* [7,8]. The information of colorants used should be listed in the ingredient part on the package of product with prefix "CI" (Color Index No.) (e.g., CI 42053 for Fast Green).

Hence, it is necessary to know the colorants applied in food and personal-care products to see whether the products fulfill the regulation or not. The analysis of colorants in food and personal-care products is typically carried out by the use of high-performance liquid chromatograph (HPLC) or gas chromatography (GC) coupled to mass spectrometry. These methods are sensitive and require small amount of sample. However, these tests are suffered from high cost and complicated sample preparation procedures. In alternative, paper chromatography can be used to identify the colorant components in a sample. It is a technique designed to separate compounds based on their differences in solubility in mobile phase (i.e., aqueous solution or organic solvent) and adsorption properties on stationary phase (i.e., the chromatography paper in this experiment). Even, the unknown colorants in a mixture can also be identified by this technique [9].

In order to identify an unknown component in a paper chromatography experiment, the final positions of each component can simply be compared. To be specific, the retention factor ( $R_f$ ) of each component can be calculated to measure their extent of movement on the paper.  $R_f$  is defined as the ratio of how far the solute travels (i.e., colorants in our experiment) on the chromatograph vs. how far the solvent front travels (Figure 2).

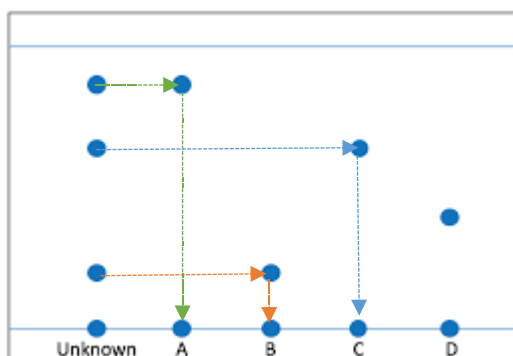


$$R_f = \frac{\text{Distance moved by the sample}}{\text{Distance moved by the solvent}}$$

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

**Figure 2.** Calculation of  $R_f$  value.

Different colorants have different solubility in the solvent. With the solvent moving upward, the more soluble colorants would migrate faster than the less soluble ones. Consequently, the more soluble colorants would have a larger  $R_f$  value than the less soluble ones. By comparing the corresponding  $R_f$  values of extracted colorants from samples with the chemical standards, the unknown components in the sample can be determined (Figure 3).



**Figure 3.**  $R_f$  value comparison.

### Intended Learning Outcomes

After the activity, students are 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 [67-63-0]
- 5 % Ammonia Solution [7664-41-7]
- Sunset Yellow (E110) [2783-94-0]
- Allura Red (E129) [25956-17-6]
- Brilliant Blue (E133) [3844-45-9]

[Tips: The colorants can be purchased from baking supply stores or chemical companies]

### *Lab preparation*

- Prepare the extraction solvent by mixing 10 mL of isopropyl alcohol and 10 mL of DI water (i.e. 1:1 mixture of isopropyl alcohol and DI water);
- Prepare the developing solvent by mixing 5 mL of isopropyl alcohol with 95 mL of 5 % ammonia solution;
- Prepare three colorant standard solutions, each by dissolving 10 mg respective standard in 1 mL of 1:1 mixture of isopropyl alcohol and DI water.

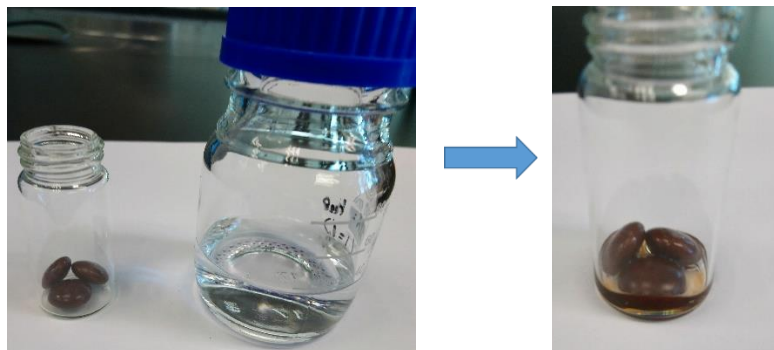


## Experimental procedures

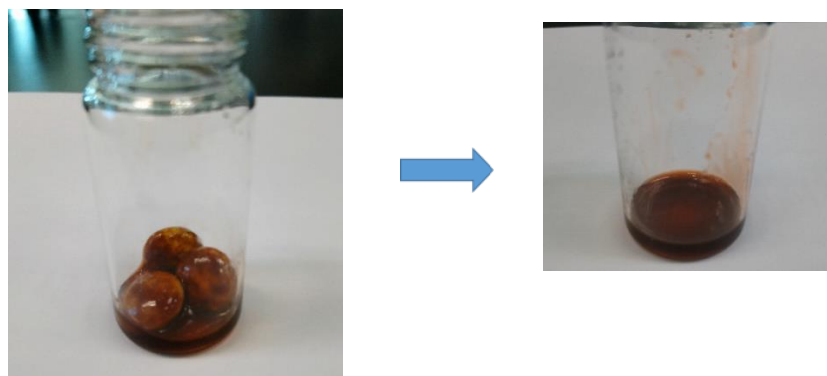
### A. Extraction of colorants from food (e.g., candies)

[Tips: Check the information of colorants used on the package of sample first]

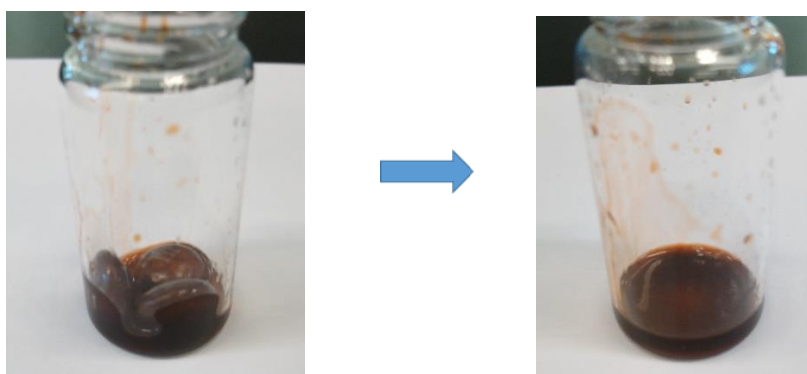
1. Place three identical candies into the vial and add 1 mL of extraction solvent.



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



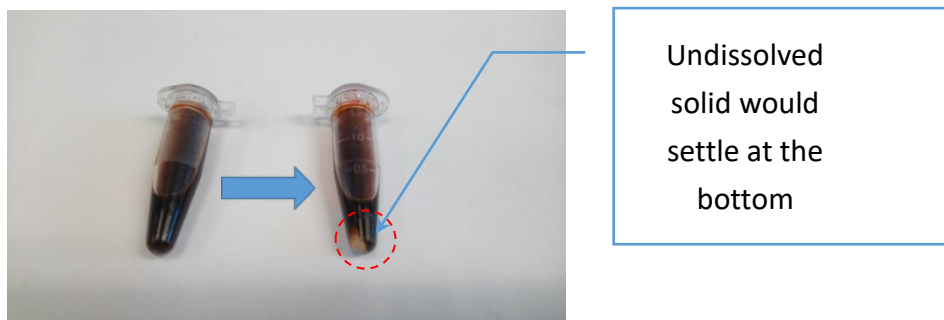
3. Add three more identical candies in the same vial and repeat step 2.



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



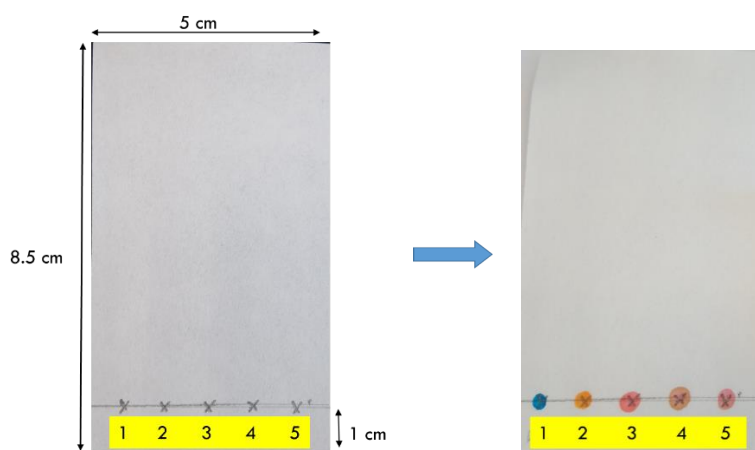
- Put the micro-centrifuge tubes into a centrifuge and spin at 13,000 rpm for 3 minutes.
- After centrifugation, a clear extracted sample is obtained.



(left: before centrifugation; right: after centrifugation)

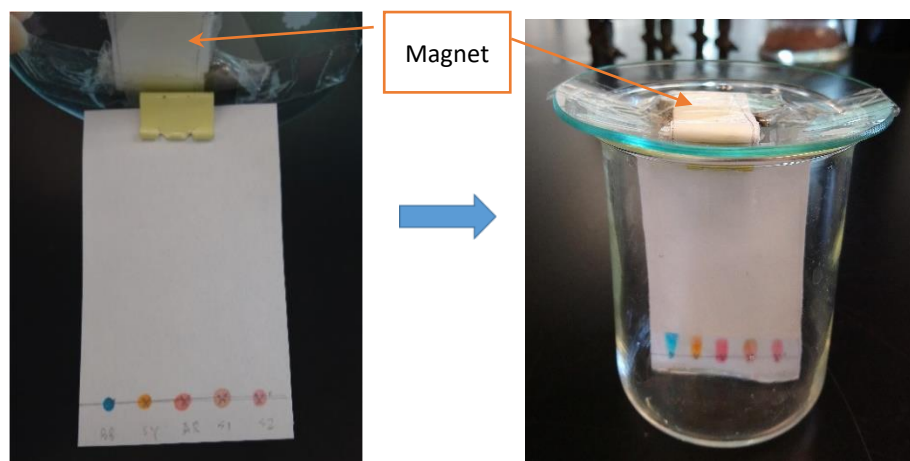
#### B. Analysis of colorants using paper chromatography

- Prepare a strip of chromatography paper with dimension of 5 cm × 8.5 cm.
- 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).
- 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.]
- Leave the paper strip stand 2 minutes for air dry.

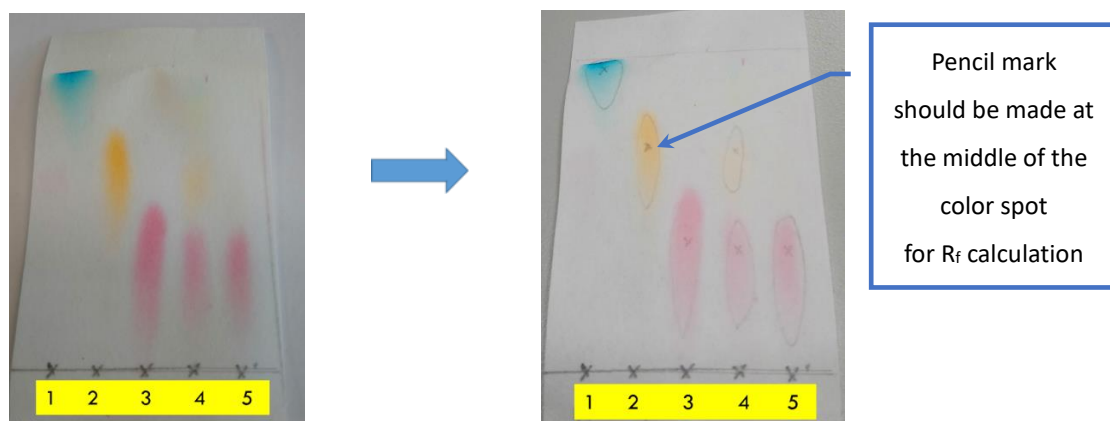


(1: Brilliant Blue, 2: Sunset Yellow; 3: Allura Red; 4: Sample A; 5: Sample B)

- Add 7 mL developing solvent into a 250-mL beaker.
- Clip the paper strip with a binder clip.
- Attach binder clip to the convex side of a watch glass with a magnet.
- 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.



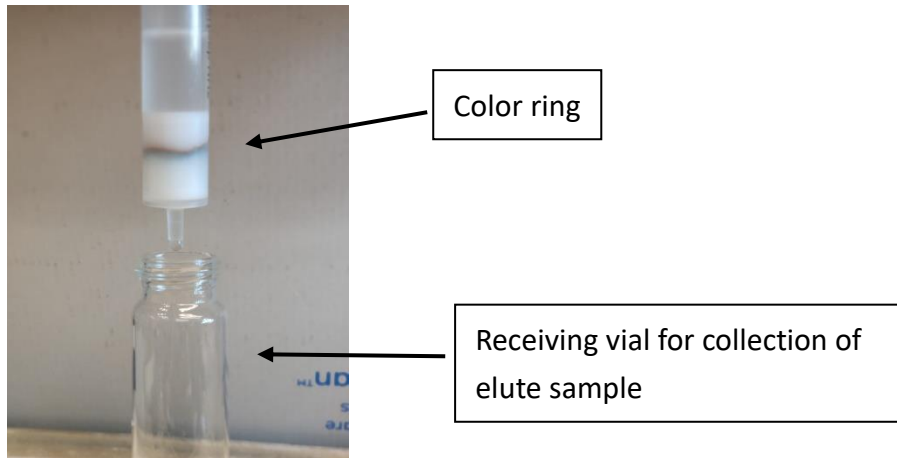
13. Compare the final positions of the colorants in the sample.
14. [Challenge] Students may calculate the  $R_f$  values of the colorants in the sample.

C. Extended/Optional Learning Activity – Analysis of colorants in soft drink and personal-care product

1. Activate the Solid Phase Extraction (SPE) column by 5 mL methanol, followed by 5 mL of 2% formic acid / DI water. (The use of SPE is to pre-concentrate the colorant(s) and remove any unwanted materials from the sample.)  
[Tips: The SPE column can be purchased from local instrument store – EasySci Instruments Co. Ltd., Tin Hang Technology Limited, and Kou Hing Hong Scientific Supplies Ltd.]



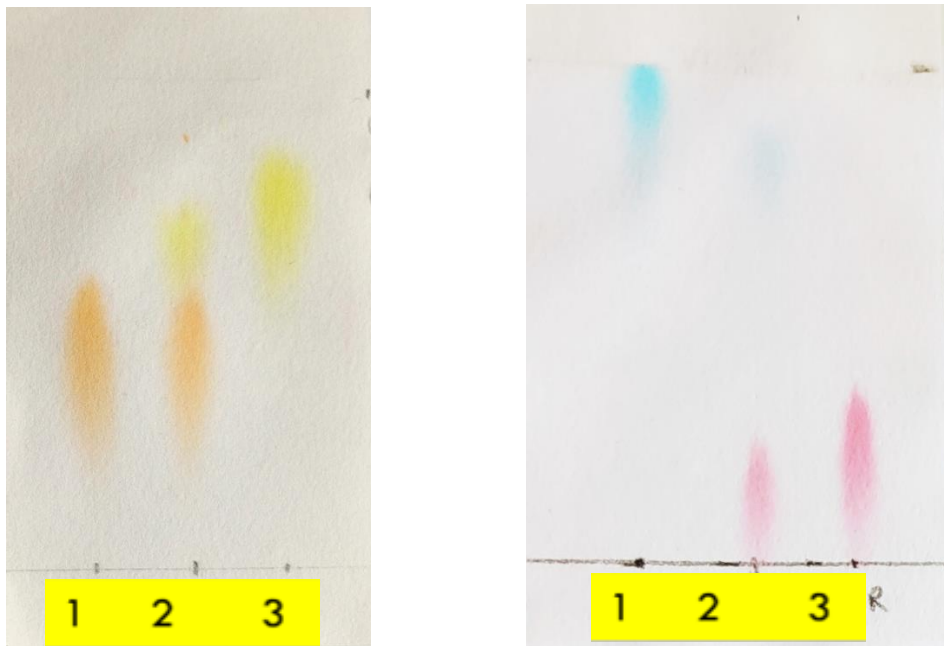
2. Add 100 mL for soft drink sample; while 30 mL for mouth wash sample.
3. Wash the column with 3 mL of DI water for 5 times.
4. Elute the colorant(s) with 5% ammonia solution.



5. Collect the colorant(s) when the color ring(s) reach the outlet of the column.
6. Around 0.5 mL of sample is collected.



7. Analyze the sample using paper chromatography.



(LEFT: Result for FANTA (Orange), 1: Sunset Yellow, 2: Sample, 3: Tartrazine)

(RIGHT: Result for Listerine, 1: Brilliant Blue, 2: Sample, 3: Allura Red)

### *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.

## **Results and Discussion**

### *Observation*

The solvent moves up the paper by capillary action and then crosses the colored spots. The spots are dissolved and move up with solvent. According to the difference in their solubility and degree of adsorption onto the chromatography paper, different colorants would migrate at different rates. As a result, the distances of travel would be different, which their  $R_f$  values would be calculated and compared. The development of the chromatogram would be fast at the beginning. However, it would slow down gradually because the moving up of solvent highly relies on the vapor content of the developing solvent. The vapor content is relatively lower at the top region of the chamber than at the bottom region and results in a slow moving up of solvent.

### *Data and data treatment*

|  | Sample A | Sample B |
|--|----------|----------|
| Color of Sample                        | Brown    | Red      |
| Number of Candies Used                 | 6        | 6        |
| Volume of Extraction Solvent Used (mL) | 1        | 1        |
| Color of the Extracted Sample Solution | Brown    | Red      |

|                       | Standard 1     | Standard 2    | Standard 3 |
|-----------------------|----------------|---------------|------------|
| Name of Colorant      | Brilliant Blue | Sunset Yellow | Allura Red |
| E Number              | E133           | E110          | E129       |
| Color of the Colorant | Blue           | Yellow        | Red        |

Distance of Solvent Front = 6.5 cm

|            | Number of Spots Developed | Distance of Travel (cm) | $R_f$ Value |
|------------|---------------------------|-------------------------|-------------|
| Standard 1 | 1                         | 6.4                     | 0.98        |
| Standard 2 | 1                         | 4.1                     | 0.63        |
| Standard 3 | 1                         | 2.2                     | 0.34        |

|          |   |              |      |
|----------|---|--------------|------|
| Sample A | 2 | 2.2 (spot 1) | 0.34 |
|          |   | 4.0 (spot 2) | 0.62 |
| Sample B | 1 | 2.1          | 0.32 |

Therefore, sample A (brown color) contains sunset yellow and allura red, while sample B (red color) contains allura red.

### *Possible measurement using advanced instrumentation/method*

Although paper chromatography is a simple and easy to use technique, it still has several limitations. First, the concentration of analytes in the spot must be high enough that each component of a mixture could be visualized after development. For example, the color intensity for tartrazine (E102) is not very high. After the chromatogram is developed, the band would spread, and the color will become even more faint and hard to be observed. Second, the solvent evaporates unevenly at the edge of the paper, always resulting in an uneven migration of the components. Third, quantitative analysis cannot be achieved, which the amount of analytes in sample is an unknown.

To overcome the above limitations, advanced chemical instrumentations can be applied, e.g., high-performance liquid chromatography coupled with a photo diode array detector (HPLC-PDA) or mass spectrometer (HPLC-MS), and gas chromatography coupled to mass spectrometry (GC-MS). The sensitivities for these techniques are high enough to detect trace amount of analytes. After the extraction and cleanup processes, the detection limit can be down to part-per-billion (ppb) level. Moreover, identification and quantification of the colorants can be achieved by knowing the respective retention time and peak area, respectively.

### **Conclusion**

By applying the paper chromatography, the colorants in food (and personal-care product) can be separated. With more than one spot displaying on the chromatography paper, it indicates that the sample contains more than one colorant. After calculating the  $R_f$  values of the sample and then comparing with those from the chemical standards, the colorants in the sample can be identified.

### **Questions and Answer**

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

*Because the ball pen or marker may contain pigment which can dissolve in the developing solvent and result as interference.*

2. Why is centrifugation needed to pretreat the sample solution?  
*Centrifugation is used to spin down the undissolved particle which would block the capillary.*
  
3. Explain how you identify different components of your unknown mixture(s) in this experiment.  
*By comparing the final positions of each component [AND/OR the  $R_f$  value] and checking the number of spots, the dyes used in the food preparation can be identified.*

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## 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. It is actually an indicator of a classification system called the Resin Identification code. This number represents the kind of plastic resin of the



product is made from in order to facilitate an easier recycling or other reprocessing.



**Table 1.** Resin Identification code of Plastics

| No. | Description                       | Examples   |
|-----|-----------------------------------|--|
| 1   | Polyethylene terephthalate (PETE) | Soft drink bottles, cooking oil containers, plastic peanut butter jars                       |
| 2   | High density polyethylene (HDPE)  | Milk jugs, shampoo bottles, cleaning product containers, detergent bottles                   |
| 3   | Polyvinyl chloride (V)            | Plastic tubing, kids' toys, plastic trays, furniture   |
| 4   | Low density polyethylene (LDPE)   | Plastic bags, Ziploc bags, buckets, squeeze bottles  |
| 5   | Polypropylene (PP)                | Food containers used for products like yogurt, sour cream and margarine, straws, bottle caps |
| 6   | Polystyrene (PS)                  | Styrofoam products, toys, carry out food containers  |
| 7   | Other                             | Polycarbonate (PC), polyamide (PA), styrene acrylonitrile (SAN)                              |

Numbers 3 and 7 generally cannot be recycled but they can be transformed into useful items like egg cartons, vents, speed bumps, cables, paneling, and more.

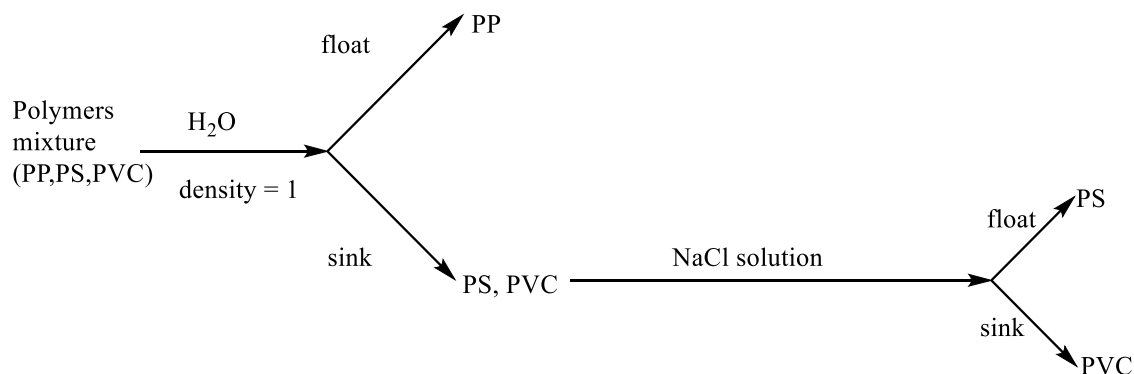
### ***Principles of the experiment***

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 2) will be separated by the physical method based on the density. The mixture is put into water. PP with lower density than water will float while PS and 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. For confirmation, advanced chemical instrumentations, e.g., infrared (IR) spectroscopy and nuclear magnetic resonance (NMR) spectroscopy, can further be applied.

**Table 2.** Density of three common types of plastics.

| Plastics                 | No. (resin code) | Density (g/mL) |
|--------------------------|------------------|----------------|
| Polypropylene (PP)       | 5                | 0.90 – 0.91    |
| Polystyrene (PS)         | 6                | 1.05 – 1.07    |
| Polyvinyl chloride (PVC) | 3                | 1.20 – 1.30    |



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

### Intended Learning Outcomes

After the activity, students are 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
- 1 x Hydrometer (Optional)

### Reagents and chemicals

- Polypropylene (PP) [9003-07-0]
- Polystyrene (PS) [9003-53-6]
- Polyvinyl chloride (PVC) [9002-86-2]
- Sodium chloride [7647-14-5]
- DI water



Figure 2. Polypropylene.



Figure 3. Polystyrene.



Figure 4. Polyvinyl chloride.

### Lab preparation

- Collect different types of plastic products made with PP, PS and PVC, and cut them into small pieces of about 5 mm x 5 mm, which will be used as sample.

### Experimental procedures

#### A. Preparation of sodium chloride solution

1. Dissolve 115 g sodium chloride in 800 mL DI water.  
(The density of this solution should be 1.1 – 1.2 g/mL.)

[Optional: Measure the density of the sodium chloride solution by a hydrometer]

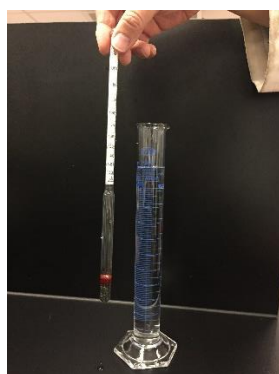


Figure 5. Hydrometer.

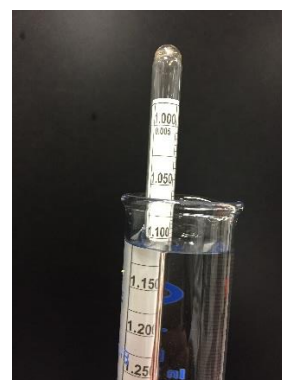


Figure 6. Measuring the density of the sodium chloride solution.

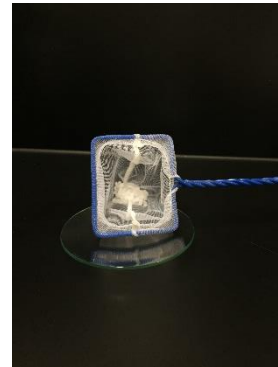
#### B. Separation of plastic standards

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.



**Figure 7.** Addition of DI water to plastic mixture.



**Figure 8.** Separation/Isolation of PP from the mixture.

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.



**Figure 9.** Addition of sodium chloride solution to plastic mixture.



**Figure 10.** Separation/Isolation of PS from the mixture.

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.

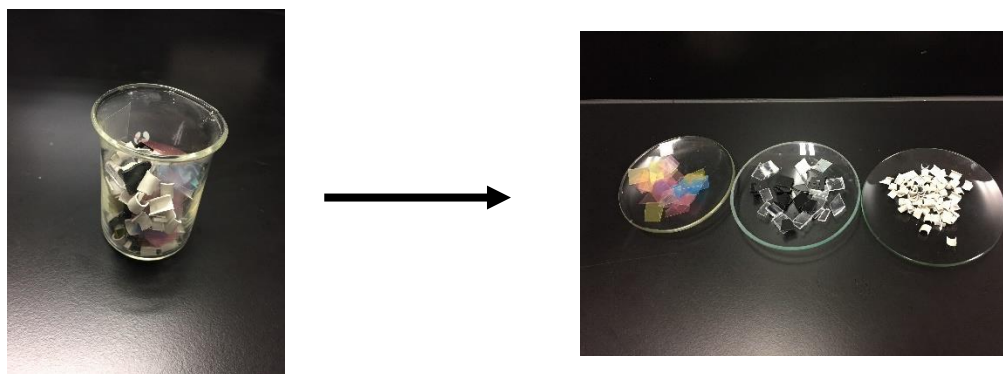
11. After 30 minutes, take out the watch glasses and let them cool down to room temperature.
12. Weigh each portion of plastics collected and compare the results with the weight of plastic standards mixed at the beginning.



Figure 11. Separated/Isolated individual plastic standards.

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.

## Results and Discussion

### *Observation*

To observe the float-sink behaviors of plastic items in liquids of known density, i.e., the heavier ones with higher density will sink, and vice versa.

|                             | Color     |
|-----------------------------|-----------|
| Sodium chloride             | white     |
| Sodium chloride solution    | colorless |
| Polypropylene standard      | white     |
| Polystyrene standard        | white     |
| Polyvinyl chloride standard | white     |

### *Data and data treatment*

Density of sodium chloride solution measured with hydrometer: 1.10 g/mL

### Separation of plastic standards

|                              | Type | Weight mixed | Weight after separation |
|------------------------------|------|--------------|-------------------------|
| Plastic floats on DI water   | PP   | 2.00 g       | 2.00 g                  |
| Plastic floats on salt water | PS   | 2.99 g       | 2.99 g                  |
| Plastic sinks in both liquid | PVC  | 3.99 g       | 3.99 g                  |

### Separation of plastic products

Weight of plastic products: 10.34 g

|                              | Type | Weight | Percentage by weight |
|------------------------------|------|--------|----------------------|
| Plastic floats on DI water   | PP   | 1.22 g | 11.80%               |
| Plastic floats on salt water | PS   | 5.06 g | 48.94%               |
| Plastic sinks in both liquid | PVC  | 4.02 g | 38.88%               |

### *Possible measurement using advanced instrumentation/method*

For confirmative identification of the plastic type after separation/isolation, advanced chemical instrumentations can further be applied:

- Infrared (IR) spectrophotometry is a technique to measure the infrared radiation absorption of plastics. Plastics with specific functional groups can be identified.
- Nuclear magnetic resonance (NMR) spectroscopy is a technique to measure the molecular structure of plastics. Plastics are dissolved in a solvent prior to study.

- Differential scanning calorimetry (DSC) is a technique to measure the physical properties of plastics upon heating, i.e., thermal properties. The respective heat capacity and glass transition temperature can be revealed.

## Conclusion

In order to achieve a more sustainable society, the effective recycling and separation of plastics are crucial. This experiment utilizes the physical property, density, of different plastic types, to differentiate them by a float-sink method. By using pure water as well as a sodium chloride salt solution with a particular concentration and specific densities, three kinds of plastics (i.e., polypropylene, polystyrene, and polyvinyl chloride) can be separated with their differences in densities. This method is efficient, low-cost, and non-toxic to human and to the environment.

## Questions and Answer

1. What is density?

*The density of a substance is its mass per unit volume.*

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

*For examples, six different kinds of post-consumer plastic, which are generally available in local wastes, namely acrylonitrile–butadiene–styrene (ABS), high density polyethylene (HDPE), polyethylene terephthalate (PET), polypropylene (PP), polystyrene (PS) and polyvinyl chloride (PVC) can be separated as well. A major source of plastic waste is the curb-side mix generated by residential households. The wastes came from cardboard (ABS), milk bottles (HDPE), drinking bottles (PET), yoghurt cups (PP), drinking cups (PS) and pipes (PVC).*

3. What is the nature of plastic?

*Plastics are generally organic polymers. The chemical properties of polymers are generally related to their synthesis, chemical structure, and molecular weight. The physical properties of polymers are generally related to their density, tensile strength, and thermal response.<sup>1</sup>*

4. Are all kinds of plastic bio-degradable?

*No. Generally, polymers that have the ester functional group, can be bio-degradable. Some polymers (bio-polymers) that are made of bio-molecular monomers, are bio-degradable.<sup>1</sup>*

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

*Same kind of plastic containers, e.g., water bottles, may not be made from the same material. The float-sink method cannot obtain a hundred percent accuracy on separations based on their chemical composition.<sup>2</sup> For recycling process, materials with the same composition must be sorted.*

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

*Most of the recycling plants and stations use automatic separation procedures that rely on the shape of the residue. Different kinds of container have different shapes. (Or: Metal waste can be sorted out by magnetic separator.) In some of the countries, most of the metallic and plastic containers are collected in the same yellow tank.<sup>2</sup>*

## **References**

1. D. E. Fagnani, A. O. Hall, D. M. Zurcher, K. N. Sekoni, B. N. Barbu, A. J. McNeil, "Short Course on Sustainable Polymers for High School Students", *Journal of Chemical Education*, 2020, 97, 2160-2168.
2. M. Barceló-Oliver, C. P. Cabello, J. Torrens-Serra, M. Miró, C. Cabot, R. Bosch, M. R. Delgado, "Scientific Activities for the Engagement of Undergraduate Students in the Separation and Recycling of Waste", *Journal of Chemical Education*, 2021, 98, 454-460.



## Module 3 – Analysis of Bacteria in Environmental Samples

### Introduction

Microorganisms (or microbes) are living organisms that are microscopic, i.e., too small to be easily seen by the naked eye. They are very diverse, including bacteria, fungi, algae, some microscopic plants, and animals, and even viruses. Microorganisms play a vital role in maintaining the Earth's ecosystem; however, some are pathogenic because they may invade and grow within other organisms and eventually cause diseases to humans, animals, or plants.

We may not see the microbes, but they are present almost everywhere; and the level of harmful microorganisms can be reduced to acceptable levels with proper hygiene techniques. This experiment is designed as a two-classroom-period activity. In this activity, we will try to reveal the existence of microorganisms from our surrounding surfaces on nutrient agar plates and see how effective the common sanitizing products, such as ethanol and bleach, in reducing the number/growth of microorganisms.

Microorganisms require nutrients to grow. Nutrient agar is generally recommended for student projects, and it can provide a general-purpose medium to support the growth of a wide range of non-fastidious bacteria and fungi. Microbes grow on solid media as colonies, which can be seen by the naked eye. Because microbial colonies on solid agar surface can be visualized, we can easily compare their microbial density without the need to use sophisticated laboratory instruments. Therefore, we can collect samples from surfaces of interest and inoculate the microbes onto nutrient agar plates using the streak plate method. Subsequently, we can estimate microbial density by colony counting using the plate count method to compare the presence of microbes on surfaces of interest.

In daily life, people usually apply different types of sanitizing products to inhibit or stop the growth of unwanted germs. Using an adapted Kirby-Bauer disk diffusion test, we can investigate the effectiveness of different sanitizing solutions against a specific type of microorganism. *S. epidermidis* is present in the normal human flora, typically on the skin and is usually non-pathogenic for healthy people; it is thus considered a safe and suitable microorganism to be used in school laboratory classes under proper supervision. On a confluent lawn of bacteria prepared by plate spreading, the antibacterial activity is observed as a clear circular zone of inhibition around the sanitizer-impregnated filter paper, indicating no bacterial growth. The size or diameter of these zones is indicative of the strength of the applied sanitizing solution.

The techniques and tests covered in this lab session are basic but very commonly used in different industries, including in the food, diagnostic microbiology, pharmaceutical and biotechnology industries.

## **Intended Learning Outcomes**

After the activity, students are 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                      Purchase from a local store
  - Household bleach solution    Purchase from a local store
  - Dettol solution                    Purchase from a local store
  - *S. epidermidis*                    [ATCC 12228]
- [Note: *Escherichia coli* can be used as alternative strain.]

## *Lab preparation*

### One day before Lab Day 1

#### A. Sterile nutrient agar solution

(Preparation: 5 mins; Sterilization cycle: 120 mins)

1. Prepare 200 mL nutrient agar solution in a 250 mL reagent bottle for each group of students, by mixing the nutrient agar powder with DI water based on the package directions.
2. Autoclave the solution at 121°C for 20 minutes in Liquid mode.  
[Note: The nutrient agar powder will not completely dissolve until it goes into sterilization cycle in autoclave.]
3. After the sterilization is complete, cool the molten nutrient agar solution to ~50°C before use, or keep it at a 60°C water bath (or oven) until further use.  
[Note: 60°C is a good temperature to keep the sterile nutrient agar solution as liquid. The agar solution will start to solidify when the temperature goes below 50°C.]  
[Note: If autoclave is not available, ready-to-use petri dish with nutrient agar can be purchased commercially.]

#### B. Prepare sterile nutrient broth

(Preparation: 5 mins; Sterilization cycle: 120 mins)

1. Prepare 100 mL nutrient broth in a 250 mL reagent bottle, by mixing the nutrient broth powder with DI water based on the package directions.
2. Autoclave the solution at 121°C for 20 minutes in Liquid mode.  
[Note: This broth is for *S. epidermidis* starter culture, it is not needed to be distributed to students.]
3. Store at 4°C until further use.

\* Aseptic technique is required for the following preparation work (C – H):

C. Prepare 1 mL of the following reagents in sterile 1.5 mL tubes

(Preparation: 15 mins)

| Reagents                    | Number of tubes required |
|-----------------------------|--------------------------|
| Sterile DI water            | 2                        |
| 75% ethanol                 | 1                        |
| 1:99 Clorox bleach solution | 1                        |
| 1:99 Dettol solution        | 1                        |

D. Prepare sterile cotton swabs

(Preparation: 5 mins)

1. Prepare six sterile cotton swabs for each group, and the swabs should be individually packed in clean resealable plastic bags.

E. Prepare sterile filter paper in a sterile petri dish

(Preparation: 5 mins)

1. Put 8 pieces of sterile filter paper (6 mm diameter) into a sterile petri dish.

F. Prepare *S. epidermidis* starter culture

(Preparation: 10 mins; Incubation: 24 hrs)

1. Add 5 mL sterile nutrient broth into a sterile bacterial culture tube.
2. Inoculate *Staphylococcus epidermidis* stock culture into the culture tube.
3. Shake the culture in a 37°C incubator at 200 rpm for 24 hours.

#### One day before Lab Day 1 or on Lab Day 1

G. Prepare nutrient agar plates

(Preparation: 10 mins; Agar solidification: 30 mins)

(This step can be done by students)

1. (Optional) Flame the mouth of the bottle containing sterile molten nutrient agar solution using a Bunsen burner, and work beside a blue Bunsen flame, if required.

[Note: This could kill off any unwanted bacteria from the mouth of the bottle and avoid agar media from getting contaminated with unwanted bacteria from the air.]

2. Lift the lid of the sterile 100-mm Petri dish slightly with one hand, and pour the agar solution (15 – 20



mL) into the dish with another hand. Close the lid and swirl the plate gently right away to obtain a uniform depth of 3 – 4 mm for the agar solution.

3. Prepare the rest of the plates. Each group requires eight agar plates, six for “Surface studies” and two for “Performance check of sanitizing products”.
4. Leave the plates on bench for at least 30 minutes to solidify.  
[Note: If you intend to use the agar plates another day, put them in a plastic bag and store at 4°C until next use.]

#### H. Prepare *S. epidermidis* diluted culture

(Preparation: 10 mins)

1. Mix 20 µl *S. epidermidis* startup culture with 980 µl sterile deionized water (1:50) into a sterile 1.5 mL tube.

### *Experimental procedures*

#### A. Surface studies (Lab Day 1, 30 mins)

Environmental sampling:

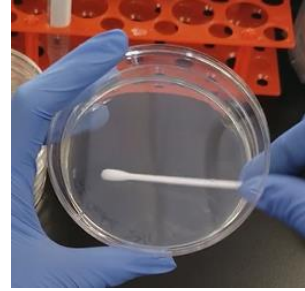
1. Provide each group with six sterile cotton swabs that are individually packed in resealable plastic bags.
2. Ask students to choose five different surfaces they are interested in testing, and do the sampling accordingly:
  - 2.1. Take out the cotton swab carefully by holding one end. Wet the other 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. Students should always prepare a control sample by dipping cotton swab with sterile water only.

Inoculation using streak plate method:

4. Apply aseptic techniques.
5. Each group needs six agar plates in this part. Have students label around the edge of the bottom (agar side) of the agar plates with group number, sample name and the date.

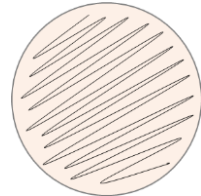


6. Ask students to inoculate samples from each cotton swab to corresponding plate, using the streak plate method. There should be six samples in total: one control and five target samples. For each sample:



- 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.

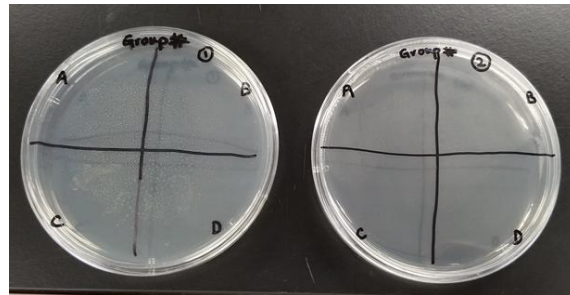


Incubation:

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 (Lab Day 1, 15 mins)

1. Distribute 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. Each group needs two agar plates in this part (two replicates). Have students label the plates as image below.

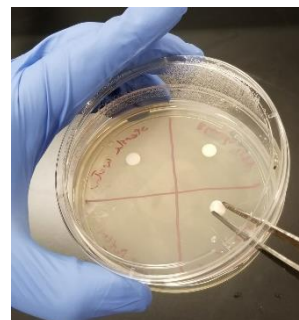


A: Sterile DI water      C: 1:99 household bleach solution  
B: 1:99 Dettol solution      D: 75% ethanol

3. Students are required to grow *S. epidermidis* using spread plate method.
  - 3.1. Apply aseptic techniques.
  - 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. Return the lid as soon as you are finished.
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. Return 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 (Lab Day 3, 15 mins)

[Note: If the class does not meet two days after, plates can be sealed with parafilm and stored upside down in a refrigerator until the following class. If the second classroom period cannot be arranged, teachers can take images for plate results and send to the students for data analysis.]

1. Two days later, students 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, students 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*

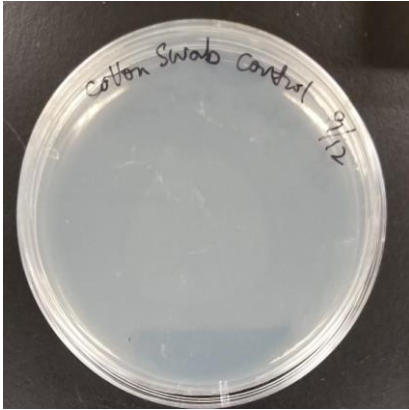
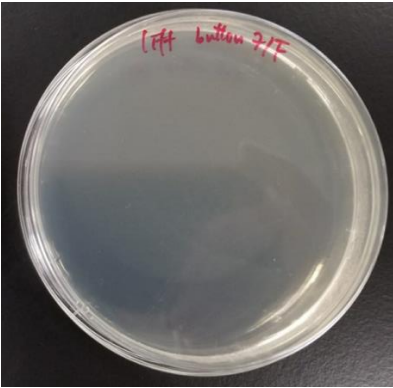
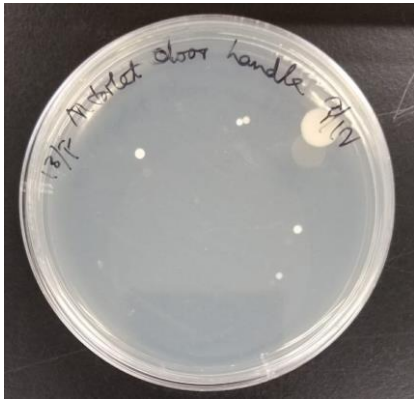
- Observe standard safety procedures for laboratory activity;
- Put on safety goggles, laboratory coats, and gloves;
- Material Safety Data Sheet (MSDS) of the chemicals are available online on [MSDSonline.com](http://MSDSonline.com);
- Wash hands thoroughly before leaving the laboratory.

### *Clean-up after experiment*


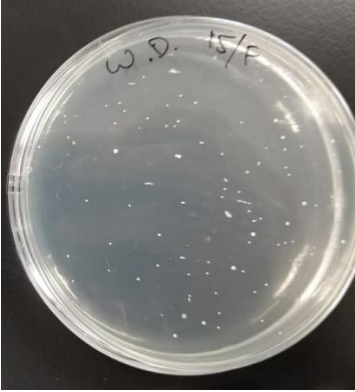
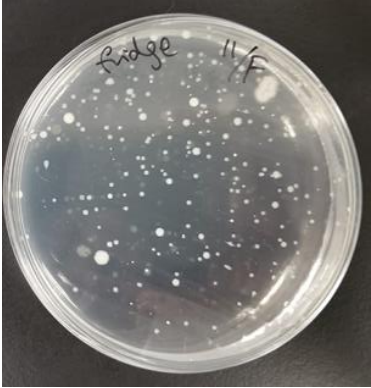
1. Teachers should properly dispose of any potentially hazardous materials, including agar plates, culture tubes, tips, tubes, swabs and spreader, with appropriate decontamination procedures.
2. Teachers should properly decontaminate equipment that will be reused.

## Results and Discussion

### A. Surface studies

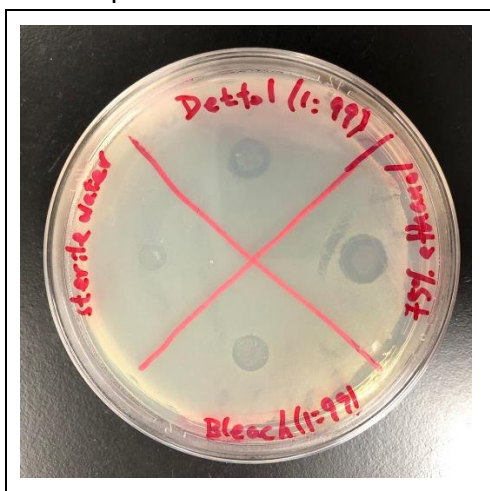
| Sample             | Image  | Colony number |
|--------------------|--|---------------|
| Control            |    | 0             |
| Life button        |   | 1             |
| Toilet door handle |  | 6             |



|                      |  |     |
|----------------------|--|-----|
| Phone set            |    | 21  |
| Water dispenser      |   | 116 |
| Door of refrigerator |  | 142 |

We collected environmental samples from some surrounding surfaces on our campus. Bacteria were found at low levels from the surfaces of the tested lift button and the tested toilet door handle. A moderate amount of bacteria was found from one phone set. Bacteria were found at high levels from the water dispenser and the door of the refrigerator. Usage and cleaning frequency, cleaning methods and personal hygiene can affect surface cleanliness. This experiment can be further applied in food industry: both food contact surfaces and non-food contact surfaces must be routinely sanitized and closely monitored to maintain good food hygiene.

## B. Performance of sanitization products



|                 | Zone of Inhibition (mm) |         |      |
|-----------------|-------------------------|---------|------|
|                 | Plate 1                 | Plate 2 | Mean |
| (A) DI water    | 6                       | 6       | 6    |
| (B) 1:99 Dettol | 9                       | 10      | 9.5  |
| (C) 1:99 bleach | 7                       | 7       | 7    |
| (D) 75% ethanol | 10                      | 12      | 11   |

After incubating the plates at 37°C overnight, we examined and measured the diameters of the zones of inhibition. The diameter of the clear zones indicates the effectiveness of the sanitizing solution. 75% ethanol, household bleach solution and Dettol solution are common surface disinfectants. Results showed that both 1:99 Dettol and 1:99 bleach had moderate inhibitory effects on the growth of *S. epidermidis*, and 75% ethanol was the most effective sanitizing agent among all. The effectiveness of the sanitizing products is dependent on the product quality and the preparation procedure, as well as the density and the types of the microorganisms. Kirby-Bauer disk diffusion test is actually more commonly used in antibiotic susceptibility test in clinical laboratories.

### *Possible measurement using advanced instrumentation/method*

Colony counter can be used to count the number of colonies.

## **Conclusion**

In the surface studies, we enabled some microorganisms in our surroundings to be visualized in the form of colonies on nutrient agar plates. Among all the surfaces we tested, the door of the tested refrigerator contained the most microorganisms. Presence of microorganism was also found on some other target surfaces, including water dispenser, toilet door handle, phone set and lift button.

To compare the effectiveness of different sanitizing products, we used an adapted Kirby-Bauer disk diffusion test. By adding a variety of sanitization products onto disks, we can measure the diameter of the zone of inhibition in the agar and determine their inhibition efficacy on bacterial growth.

From this experiment, we revealed the presence of microorganisms from our surroundings, and we believe that common disinfection methods could reduce the levels of certain microorganisms.

### Questions and Answer

1. According to your results, which surface was the dirtiest?  
*The surface of the refrigerator door was the dirtiest among the tested surfaces.*
2. Among the three given sanitizing solutions, which one(s) is/are the strongest disinfectant(s) against *S. epidermidis*?  
*75% ethanol is the strongest disinfectant against S. epidermidis among the three given solutions.*
3. What type(s) of microorganisms do you expect to grow on your agar plates?  
*Bacteria, e.g., staphylococci, micrococci, E. coli*  
*Fungi, e.g., yeasts and molds*
4. Why do we need to apply aseptic techniques and use sterile items during some steps of the experiment?  
*To minimize the risk of contamination / To prevent to the growth of unwanted microorganisms on the agar plates.*
5. Suggest two personal hygiene measures to prevent infectious disease.  
*Washing hands before meals, washing hands after using the washroom, or other appropriate answers*

### References

1. [https://bio.libretexts.org/Bookshelves/Microbiology/Book%3A\\_Microbiology\\_\(Boundless\)](https://bio.libretexts.org/Bookshelves/Microbiology/Book%3A_Microbiology_(Boundless))

## 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.



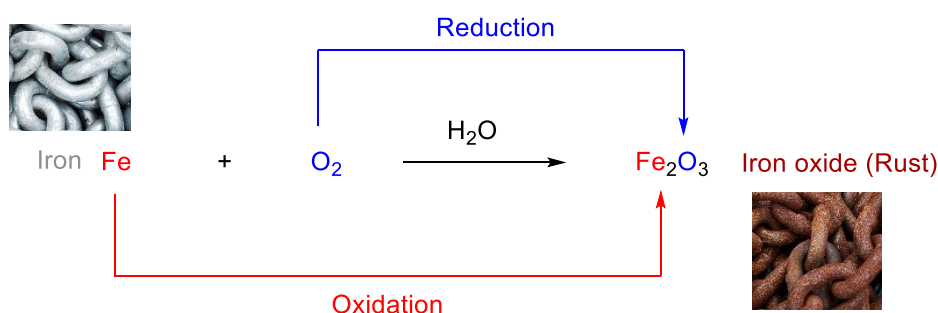
Ethanol produced by fermentation of sugars can also be used as a fuel or a chemical feedstock. In Brazil, there is an extensive programme of alcohol production from sugar cane and the resulting “GASOHOL” (a mixture of petrol and ethanol) is used as a fuel for motor vehicles. Chemical companies in the West are also interested in using the ethanol produced from sugar cane in Brazil as a chemical feedstock for manufacturing of plastics. Although it is not economical at this moment, it may become more practical as the production costs fall. The “wine lake” (supply surplus of wine produced in the European Union) has also been considered as another source of alcohols that could be distilled to produce alcohols as motor fuel and a chemical feedstock for manufacturing of plastics.

Ethanol ( $\text{CH}_3\text{CH}_2\text{OH}$ ) can also be oxidized to acetic acid ( $\text{CH}_3\text{COOH}$ ) by a genus of bacteria called vinegar bacteria, also known as acetic acid bacteria (AAB). Acetic acid is the major component in vinegar. Therefore, vinegar can be produced from any alcoholic materials from alcohol-water mixtures to various fruit wines. Acetic acid would be produced by a two-step bioprocess. As shown below, fermentable sugars are first transformed into ethanol by the action of yeasts. In the second step, AAB oxidize ethanol to acetic acid via an aerobic process. AAB are well known for their ability to spoil wines because they can produce large amounts of acetic acid from ethanol and other compounds present in wines.

| Anaerobic  | Aerobic  |
|--|--|
| Glucose $\xrightarrow{\text{Yeast}}$ $2\text{C}_2\text{H}_5\text{OH}$<br>Ethanol | $\xrightarrow{\text{AAB}}$ $2\text{CH}_3\text{COOH} + 2\text{H}_2\text{O}$<br>Acetic acid<br>(Vinegar) |

### B. Redox reaction

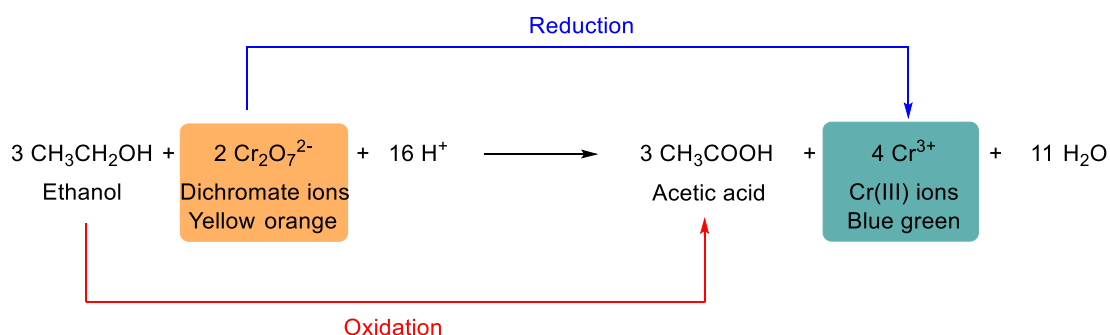
An oxidation-reduction reaction (also known as a redox reaction) is a type of chemical reaction that involves a transfer of oxygen. Redox reactions can be observed in our vicinity. For example, rusting of iron is a redox reaction in which iron (Fe) is oxidized to iron oxide ( $\text{Fe}_2\text{O}_3$ ) by oxygen in the presence of water.



### C. 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 a method based on the oxidation-reduction (redox) reaction between ethanol ( $\text{CH}_3\text{CH}_2\text{OH}$ ) and dichromate ions ( $\text{Cr}_2\text{O}_7^{2-}$ ). As shown in the redox reaction below, ethanol in the alcoholic beverages will be oxidized to acetic acid ( $\text{CH}_3\text{COOH}$ ) by a dichromate solution (a yellow orange color solution) under an acidic condition. Meanwhile dichromate ions will be reduced to Cr(III) ions ( $\text{Cr}^{3+}$ ), which is in blue green color. The amount of Cr(III) ions generated can be determined by measuring the absorbance of yellow light (wavelength = 580 nm) with a simple colorimeter.

The redox reaction between ethanol and dichromate ions:

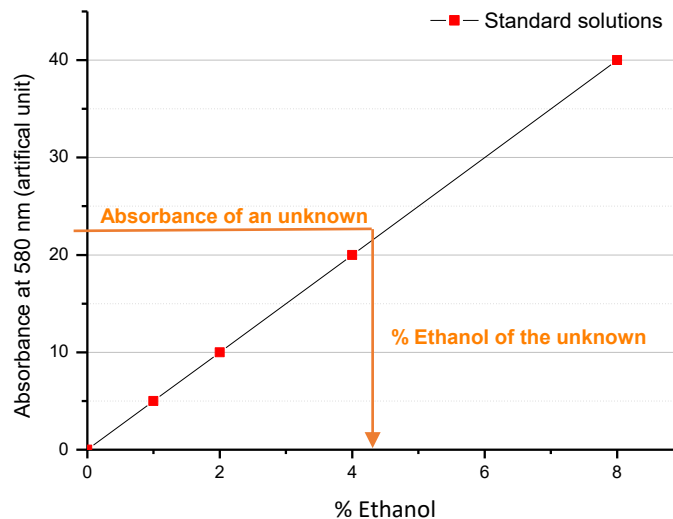


In this case, dichromate ions are in excess and ethanol is the limiting reagent. The generation of Cr(III) ions is directly proportional to the amount of ethanol in the solution. The amount of Cr(III) ions is proportional to the absorbance of the yellow light (wavelength = 580 nm) based on the Beer's law:

$$A = abc$$

where A = absorbance, a = absorptivity, b = light pathlength, and c = concentration.

Therefore, the amount of ethanol is directly proportional to the absorbance at 580 nm. 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.



[Remark: To make the concept simple, the dichromate ions can be regarded as a color indicator that there will be a color change upon reacting with ethanol.]

[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, students are 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)
- 5 x 100 mL volumetric flasks
- 1 x 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)



Figure 1. Glass apparatus

### *Reagents and chemicals*

- Glucose [50-99-7]
- Yeast (wine production)
- Yeast extract [8013-01-2]
- Ethanol [64-17-5]
- Potassium dichromate [7778-50-9]
- Sulphuric acid [7664-93-9]

### Lab preparation

- Prepare a set of ethanol solutions in 100 mL volumetric flasks.

|          | 0% (Blank)                                  | 1%   | 2%   | 4%   | 8%   |
|----------|---|------|------|------|------|
| Ethanol  | 0 mL  | 1 mL | 2 mL | 4 mL | 8 mL |
| DI water | Make up to the mark of the volumetric flask |      |      |      |      |

- Prepare acidified potassium dichromate solution by dissolving 1.5 g potassium dichromate in 100 mL sulphuric acid of 3 M.

### Experimental procedures

[Remarks: This experiment involves two parts: (A) Wine-making using fermentation (it is suggested to be a demonstration carried out by the teacher. The reaction takes about 2 hours, but it is not necessary to wait until the completion of the reaction. Students should be able to smell the ethanol produced by fermentation after 30 minutes); and (B) Analysis of ethanol in alcoholic beverages (~1 hour). Depending on the time of the laboratory class, teachers can do one or both parts of experiments with students.]

#### 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:

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

|                               | 0%<br>(Blank) | 1%          | 2%          | 4%          | 8%          |
|-------------------------------|---------------|-------------|-------------|-------------|-------------|
| Ethanol solution              | 200 $\mu$ L   | 200 $\mu$ L | 200 $\mu$ L | 200 $\mu$ L | 200 $\mu$ L |
| Potassium dichromate solution | 2.0 mL        | 2.0 mL      | 2.0 mL      | 2.0 mL      | 2.0 mL      |

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.

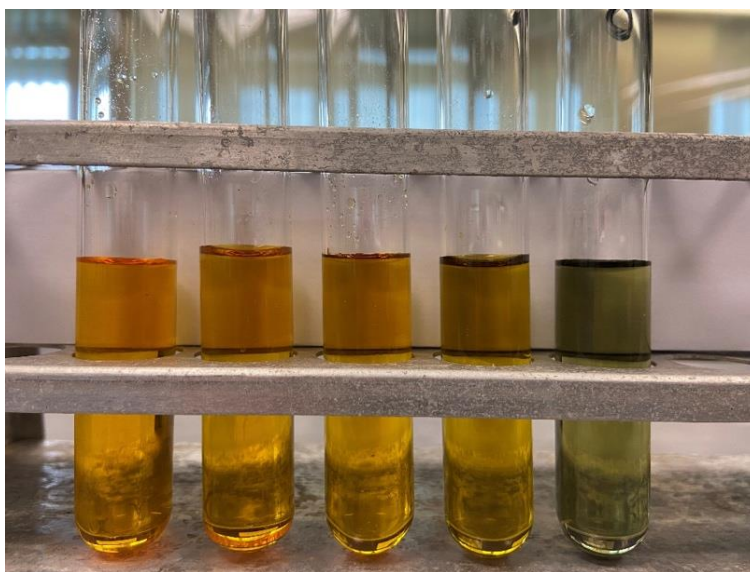


For iOS device

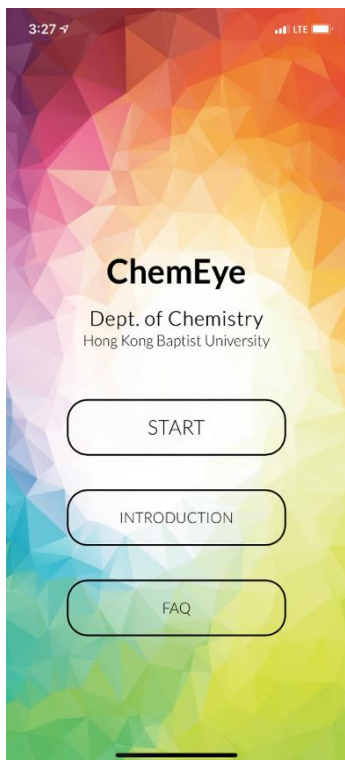


For Android device

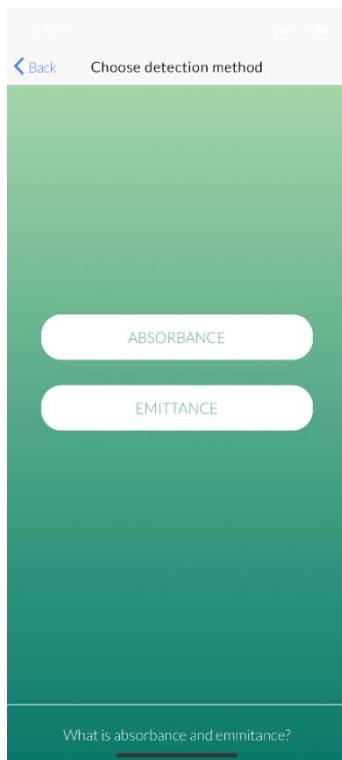
8. Place the test tubes in front of white background.



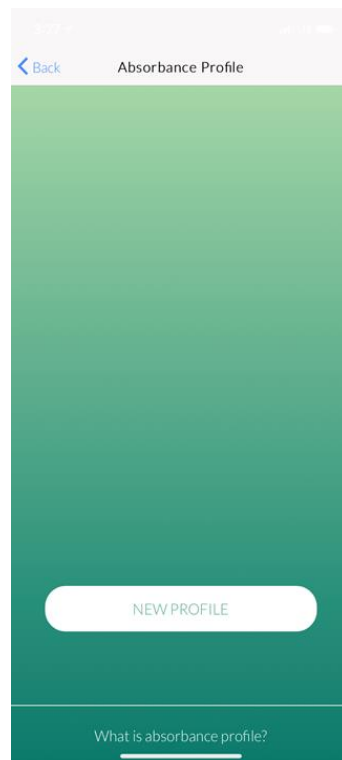
9. Follow the procedures to create the calibration curve and detect the sample:



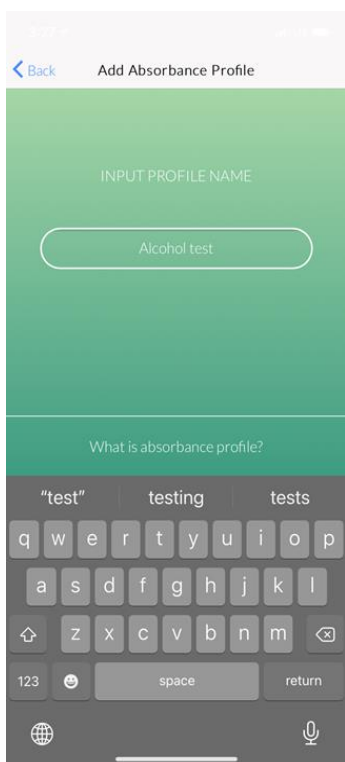
1. Open the App and press "START"



2. Choose "ABSORBANCE"



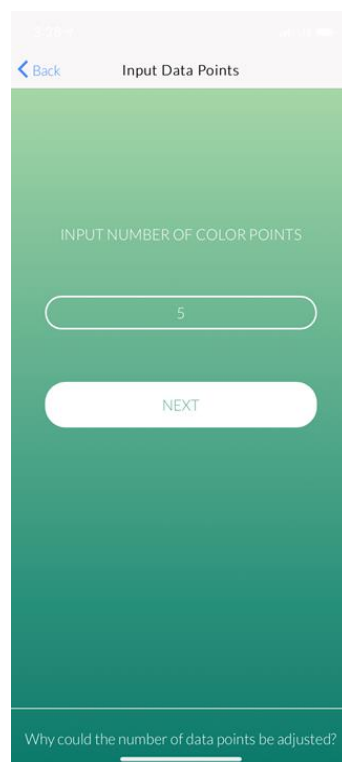
3. Click on "NEW PROFILE"



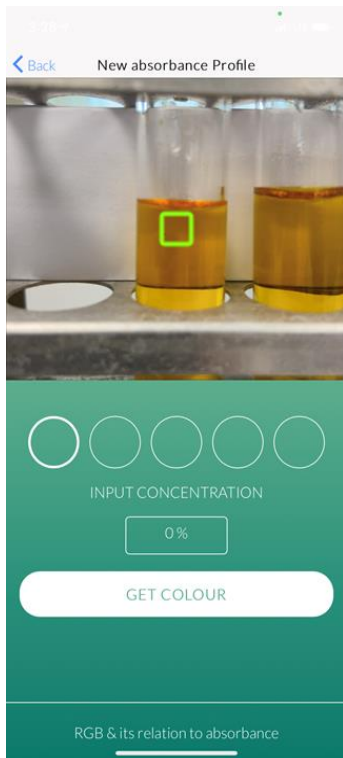
4. Input the profile name



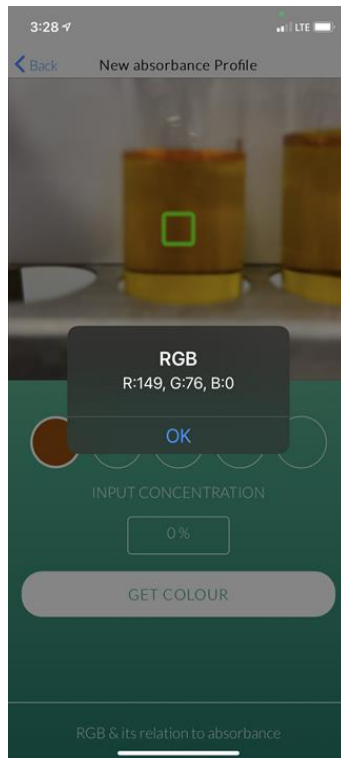
5. Input the unit as Percentage %



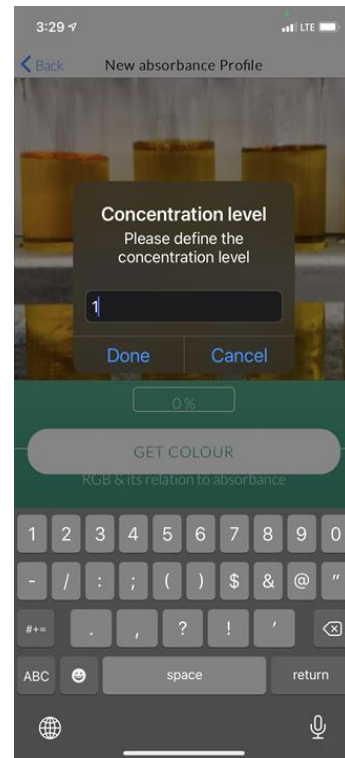
6. Input the number of color points as 5



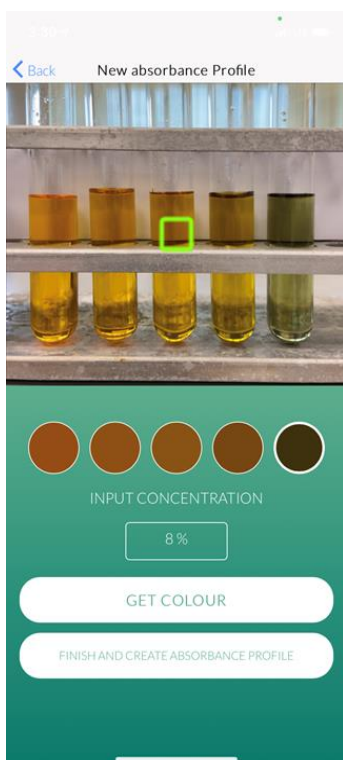
7. Place the green square on the standard solution and press "GET COLOR"



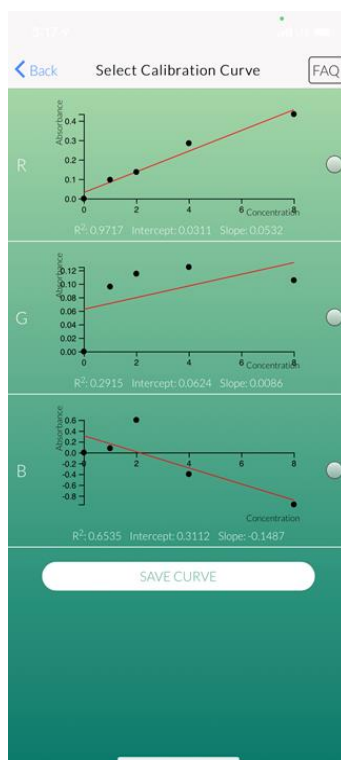
8. RGB value obtained



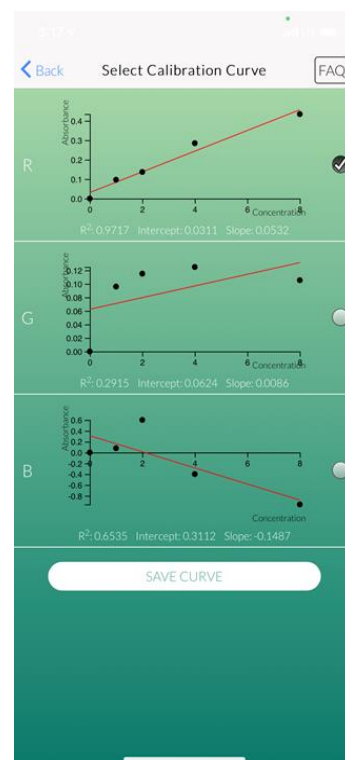
9. Enter the respective concentration



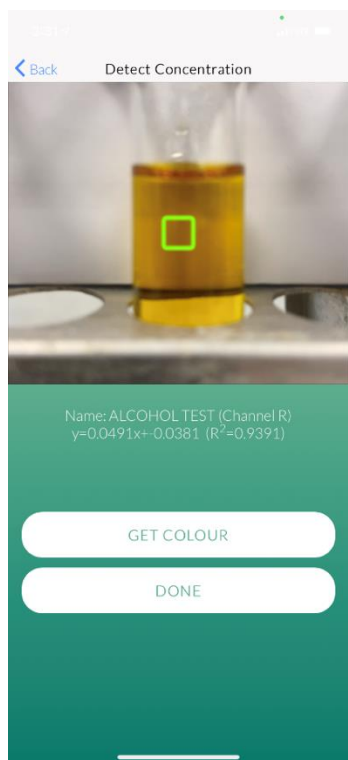
10. Repeat all standard solutions. Click on "FINISH"



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



12. Red curve selected in this experiment.



13. The best straight line equation with  $R^2$  shown



14. "DETECT" the sample



15. Detected concentration 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.

## **Results and Discussion**

### *Observations*

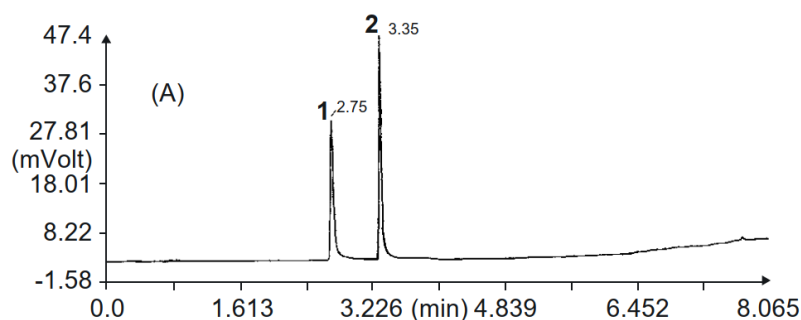
- A. Wine-making using fermentation
  1. A creamy yellow solution is obtained when dissolving sugar and yeast in DI water.
  2. Bubbles are generated during the fermentation.
- B. Analysis of alcohol in beverage using ChemEye (smartphone colorimetry)
  1. Blank solution is in yellow color; Ethanol containing solutions are in yellow to green color.

### *Data and data treatment*

(as shown in the experimental procedure)

### *Possible measurement using advanced instrumentation/method*

Capillary gas chromatography is a rapid method for determination of ethanol in alcoholic beverages (Reference 3). It takes only 8 minutes to complete a sample analysis for the determination of ethanol content in a beverage sample. A sample solution is mixed with adequate amount of internal standard solution, and injected into a capillary gas chromatography. The study method can be applied to alcoholic beverages with different alcoholic contents, and with the advantages of simple sample pre-treatment procedures.



### **Conclusion**

Through the experiment, students can acquire the concept of fermentation from sugar to generate alcohol for measurement, i.e., ethanol is reacted with acidified potassium dichromate solution. The data obtained in the experiment can be checked against regulatory controls or beverage's label, which helps to develop the student awareness of alcohol in beverage and also interest in food analysis.

### **Questions and Answer**

1. What is the role of yeast in fermentation?

*Yeast converts sugar into ethanol and carbon dioxide during primary fermentation in brewing.*

2. What conditions may affect the fermentation reaction?

*Fermentation is one of the biological reactions. Therefore, the temperature will affect the reaction as enzymes tend to have an optimum temperature. Under a higher or lower temperature, enzymes do not work efficiently and the reactions will not happen.*

*The presence of air (oxygen) will also have an effect on this reaction. The fermentation reaction occurs in anaerobic conditions. The presence of oxygen can affect the rate of reaction.*

3. Give examples of redox reactions in daily life.
  - *Respiration*
  - *Combustion*
  - *Photosynthesis*
  - *Corrosion*

## References

1. Sandip V Sumbhate, Satish Nayak, Damodar Goupale, Ajay Tiwari, Rajesh S Jadon, Journal of Analytical Techniques, Vol. 1, No. 1 (2012).
2. "Detection in daily life: Using Google Science Journal and ChemEye", [https://cloud.aristo.com.hk/STEM/Science/resources/item\\_35\\_science/scient\\_ST\\_SExSTEM\\_detection\\_final\\_e\\_stu.pdf](https://cloud.aristo.com.hk/STEM/Science/resources/item_35_science/scient_ST_SExSTEM_detection_final_e_stu.pdf)
3. Mei-Ling WANG, Youk-Meng CHOONG, Nan-Wei SU and Min-Hsiung LEE, "A rapid method for determination of ethanol in alcoholic beverages using capillary gas chromatography", Journal of Food and Drug Analysis, Vol. 11, No. 2, 2003, Pages 133-140.

## Data Sheet

### Qualitative analysis by ChemEye

| Sample | Description             | Labeled content (%) | Ethanol detection result by ChemEye (%) | R,G,B Line selection |
|--------|-------------------------|---------------------|---|----------------------|
| 1.     | San Miguel Pale Pilsner | 5.0                 | 4.5                                     | R                    |
| 2.     |                         |                     |   |                      |
| 3.     |                         |                     |   |                      |

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

### Introduction

Slime-making is a fun science activity which provides an opportunity for students to learn about viscosity, polymers, and chemical bonds. Slime is a gooey substance which you can make from common household products. The starting materials, such as glue and water, are in a liquid form. Upon the addition of borax solution and well-mixing, a sticky mass, known as slime, is formed [1].

In this experiment, polyvinyl alcohol (PVA) and borax (sodium tetraborate) are used to produce PVA-borate hydrogels – “slime”. Borax allows the formation of cross-linkages that connect two polymer chains of PVA together through covalent bond. With the increase of the number of cross-linkages in the structure, the polymer chains will be held tightly, making the slime to be stiffer and more viscous.

We encounter polymers every day, such as cellulose in plants, keratin in hair and nails, and all types of plastics in consumer products. Glue is one of the examples of a polymer composed of long chains of polyvinyl acetate (PVAc) (Figure 1). It is a synthetic polymer prepared by the polymerization of vinyl acetate monomer. Polymer is a large molecule with lots of repeating units. Typically, PVAc in glue is made up of ~1800 repeating units, which can be further reacted with water to some extent replacing some of the acetate groups with an alcohol (-OH), i.e., PVAc is partially converted to PVA.

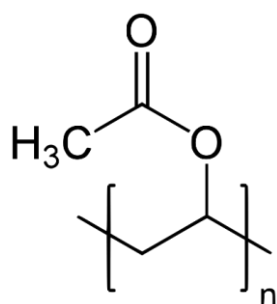


Figure 1. Structure of PVAc.

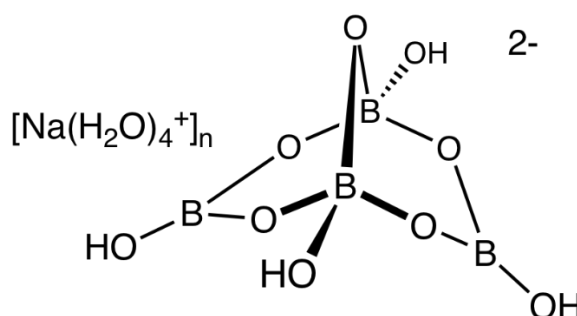


Figure 2. Structure of borax.

Borax –  $\text{Na}_2[\text{B}_4\text{O}_5(\text{OH})_4]\cdot 8\text{H}_2\text{O}$ ; sodium tetraborate – is a powdery white mineral (Figure 2), which has been used as a cleaning product for decades. It can be used to get rid of stains, mold and mildew around the house, and to kill insects such as ants.

When a 2% borax solution is added to a mixture of water and glue in 1:1 ratio, a highly viscous and very resilient form of slime will be formed (Figure 3). In aqueous solution,

borax dissociates to form tetrahydroxyborate ion,  $B(OH)_4^-$ . Mixed with PVA, the  $B(OH)_4^-$  ion forms cross-linkages between the -OH groups on adjacent polymer chains. Indeed, the cross-linkages are relatively weak which can break and reform continuously. With this property, the resulting slime are somewhat solid and somewhat liquid. Moreover, the degree of cross-linking can be varied by the borate concentration that the texture of the resulting slime can be quite different.

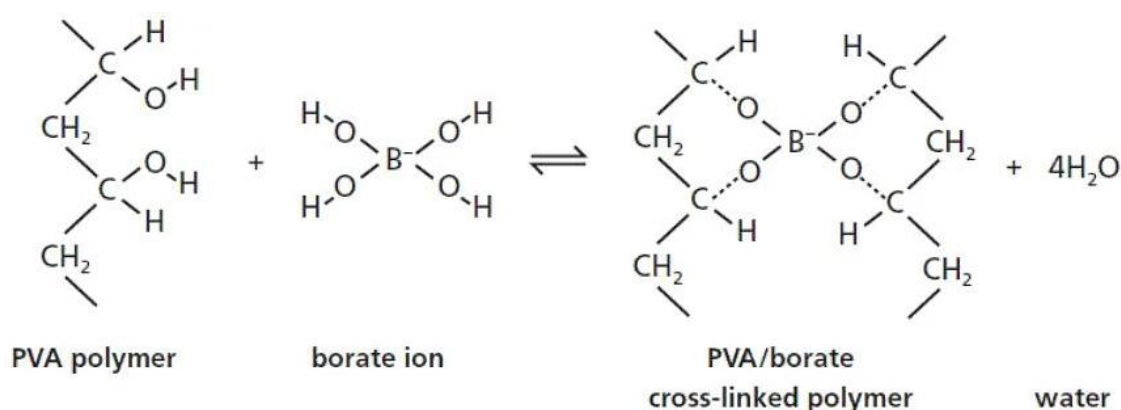


Figure 3. Formation of slime through cross-linkages.

However, slime safety has been gaining substantial attention from the public for years. Borax is a highly alkaline and is also a known eye, nose, and respiratory tract irritant. The ingestion of borax can also lead to reproductive issues. Thus, the identification and quantification of borax in slime products is necessary to safeguard the public health.

In general, borax is determined quantitatively using inductively coupled plasma-mass spectrometry (ICP-MS) [2]. However, it requires specific extraction method for sample pretreatment, and the operation cost are also expensive. To suit the secondary school environment, a simple and sensitive approach to detect borax in slime products using turmeric powder is developed and applied as a screening test [3]. 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 (Figure 4). The reaction is highly sensitive that trace amount of borax can be detected.

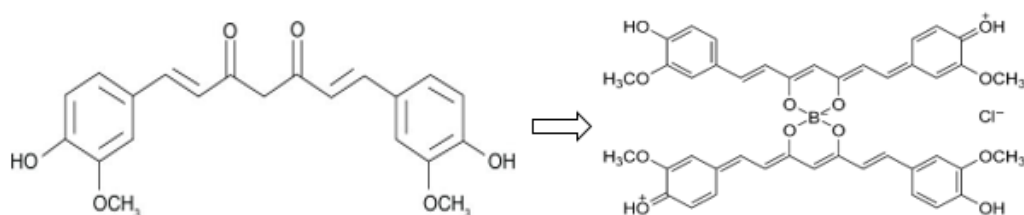


Figure 4. Reaction between the curcumin and borax to be rosocyanine.



For quantification, acid-base titration is herein used to determine the amount of borax in the slime. Firstly, standardized HCl will be added in excess to neutralize the  $B(OH)^4-$  to destroy the cross-linkages among the polymer chains. Upon the complete reaction with all the borax in the slime, the amount of HCl remained is then determined using titration with standardized NaOH. Methyl red is used as the indicator in this titration, changing its color from red to yellow at the pH corresponding to the inflection point. Finally, the amount of borax can be calculated.

In this laboratory activity, students will investigate the cross-linkage and observe how it changes the physical properties of a polymer, as well as qualitative test of borax using natural color indicator and quantitative determination of borax in slime by acid-base titration. All the above methodologies are feasible and practical for secondary school classroom instruction.

### **Intended Learning Outcomes**

After the activity, students are 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 [64-17-5]
- Hydrochloric acid (HCl) [7647-01-0]
- Methyl red indicator [493-52-7]
- Sodium hydroxide (NaOH) [1310-73-2]
- Borax Purchase from a local store
- White Glue Purchase from a local store
- Turmeric powder Purchase from Asian grocery store

### *Lab preparation*

#### 1. Preparation of 2% Borax solution

Dissolve 2 g of borax in 50 mL DI water. Then, transfer the solution into a 100 mL volumetric flask and dilute to the mark with DI water.

#### 2. Preparation of turmeric indicator

Weigh 0.5 g of turmeric powder on an analytical balance and transfer the yellow powder into a 100 mL beaker. 50 mL of 70% ethanol is added for dissolution.

#### 3. Preparation of 0.1 M NaOH solution

Dissolve 0.4 g of sodium hydroxide in 50 mL DI water. Then, transfer the solution into a 100 mL volumetric flask and dilute to the mark with DI water.

#### 4. Preparation of 0.1 M HCl solution

Dilute 8.3 mL of conc. HCl to 700 mL DI water. Mix well and allow to cool to room temperature. Dilute to the mark with DI water in a 1 L volumetric flask. Keep the solution for at least one hour prior to use.

### *Experimental procedures*

#### A. Slime-making (40 mins)

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.

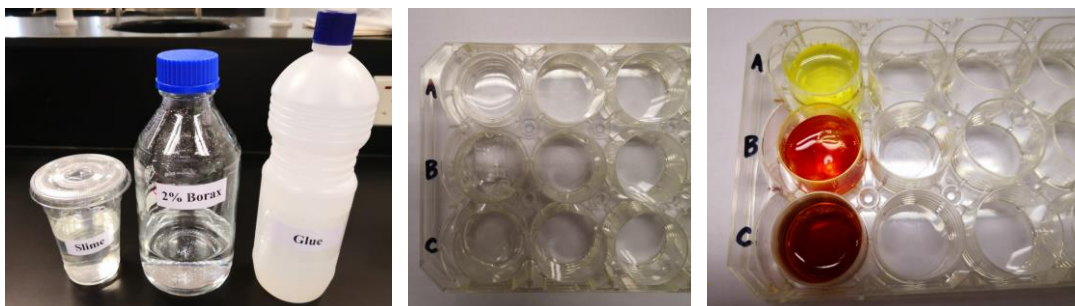
[Tips: DO NOT add too much borax solution. Stop adding the borax solution when there is still a little glue-water mixture left in the bottom of the cup.]

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 (10 mins)

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 (40 mins)

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.

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



### *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.

## **Results and Discussion**

### *Observation*

- Reddish color is observed when turmeric indicator is added to samples containing borax.

|        | Color   |
|--------|---------|
| Well A | Yellow  |
| Well B | Reddish |
| Well C | Reddish |

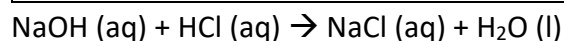
\* Curcumin is the yellow natural pigment from turmeric. Curcumin forms colored complexes of reddish with borax.

- At the end point of the titration for the determination of borax content, the color of the solution changes from red to yellow.

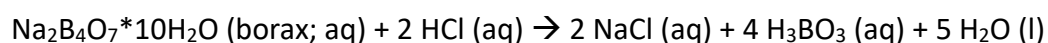
## Data and data treatment

### Analysis of Borax in slime using titration

|                          |         |
|--------------------------|---------|
| Mass of slime (g)        | 52.1136 |
| Volume of HCl added (mL) | 15.00   |
| Initial volume (mL)      | 10.00   |
| Final volume (mL)        | 17.55   |
| Volume of NaOH used (mL) | 7.55    |



∴ Mole ratio of NaOH : HCl = 1:1



∴ Mole ratio of HCl : Borax = 2:1

Molar mass of borax = 381.38 g/mol

$$\text{Borax} = \frac{\text{mass of borax}}{\text{mass of slime used}} \times 100\%$$

$$\text{Borax} = \frac{\text{number of mole of borax} \times 381.38}{\text{mass of slime used}} \times 100\%$$

$$\text{Borax} = \frac{\text{number of mole of HCl used} / 2 \times 381.38}{\text{mass of slime used}} \times 100\%$$

$$\text{Borax} = \frac{(\text{initial mole of HCl} - \text{final mole of HCl}) / 2 \times 381.38}{\text{mass of slime used}} \times 100\%$$

$$\text{Borax} = \frac{(0.1 \times 15/1000 - 0.1 \times \text{volume of NaOH used}/1000) / 2 \times 381.38}{\text{mass of slime used}} \times 100\%$$

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

$$\text{Borax} = 0.3\%$$

### Possible measurement using advanced instrumentation/method

Borax, in terms of boron, can be determined quantitatively in soil, water, and biological samples using spectrometric and colorimetric methods. For spectrometric methods, the application of inductively coupled plasma-mass spectrometry (ICP-MS) provides the

highest sensitivity and lowest detection capability for boron determination. ICP-MS is the most sensitive method currently available having a detection limit of 0.01 mg/L. Filtration and acidification with a proportion of 1 mL of concentrated HNO<sub>3</sub> per 100 mL of sample are commonly used for sample preparation, only dissolved boron is analyzed and introduced to ICP-MS [4].

## Conclusion

A solution of polyvinyl alcohol (glue) can be made into slime by adding borax solution, which creates cross-linkages between polymer chains. In this activity, some interesting texture properties of the slime are investigated. Further, a fast, low-cost, and sensitive screening test for borax using turmeric powder is developed and applied for daily life application without any advanced instrumentation. Meanwhile, students should have learnt the testing protocol of determining the amount of borax by acid-base titration.

## Questions and Answer

1. Is slime a liquid or solid? Give your reasons below.  
*Slime is neither liquid nor solid. It presents with the properties of both phases and is actually classified as Newtonian fluid.*
2. Describe the properties of slime and how it feels in your hands.  
*The slime feels slightly moist and sticky. It flows slowly when left on the table. It is soft and has some force resistance when pulling the slime apart.*
3. What are some of the polymers that you encounter every day?  
*Sandwich bags, carpets, nylon stockings, clothing made from synthetic fibers, milk cartons, etc.*

## References

1. Brian Rohrig, Chemmatters, 12, 13-16, 2004.
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3. Nanang Rahman, J. Phys:Conf.Ser., 1363, 2019.
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## 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. They are not sustainable because of severely incurred pollutions and global warming. Scientists have been searching for alternative renewable energy sources that are pollution-free and cheap but can still provide high efficiency. In today's society, an eco-friendly environment is of much concern; and hence, solar energy has become one of the best viable options because it is renewable and does not produce any pollutants. It is also known to be one of the cleanest energy sources. 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. Dye-sensitized solar cells (DSSC) have attracted much attention 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.

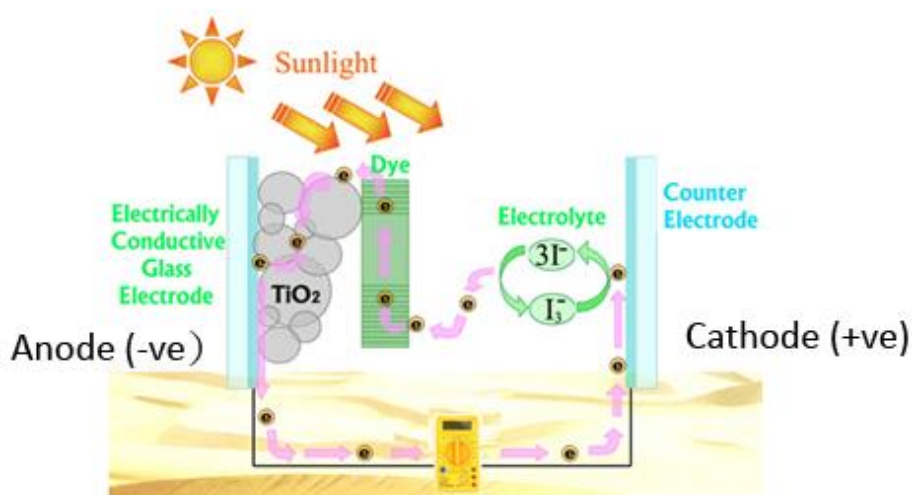


Figure 1. A schematic diagram of a typical dye-sensitized solar cell.

The working principle of a dye-sensitized solar cell involves three steps (Figure 1):

1. A dye coated onto a TiO<sub>2</sub> layer is used to absorb sunlight, which transforms solar energy into chemical energy to promote an electron from its ground-state to the excited-state;
2. The excited electron is injected from the dye into the TiO<sub>2</sub> layer and reaches the electric contact travelling to the external circuit; and
3. The oxidized dye is finally reduced to its original state by the I<sup>-</sup>/I<sub>3</sub><sup>-</sup> redox couple in electrolytic solution carrying the electrons arriving at the counter electrode and completing the circuit.

Each cell is like a “sandwich”, in which two conducting glasses are overlapped. The photoanode is coated with the layer of TiO<sub>2</sub> adsorbed with the photosensitizing dye and the cathode is coated with carbon which serves as a catalyst in order to enhance the contact between the electrolytic solution (iodide/triiodide) and the glass electrode. Solar cell generates electricity when light shines on it.

### **Intended Learning Outcomes**

After the activity, students are 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 50 mL beaker
- 4 x Wires with alligator clips
- 1 x Stopwatch with alligator clip
- 1 x Watch glass

\* FTO (fluorine doped tin oxide) conductive glass is used, instead of ITO (indium tin oxide) conductive glass for the preparation of dye-sensitized solar cells. ITO electrical properties can be degraded in presence of oxygen at relatively high temperature (i.e., around 500 °C) while FTO is much more stable in such conditions.

### *Reagents and chemicals*

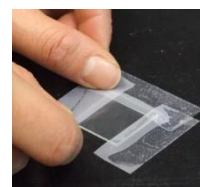
- Ethanol [64-17-5]
- Potassium iodide [7681-11-0]
- Iodine [7553-56-2]
- Ethylene glycol [107-21-1]
- DI water
- Blueberries (frozen fruit bought from supermarket)
- TiO<sub>2</sub> paste\*\* [13463-67-7]

\*\* TiO<sub>2</sub> paste is purchased from Man Solar Company: <https://www.mansolar.nl/home>

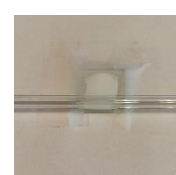
### Lab preparation

#### A. Coated titanium dioxide (TiO<sub>2</sub>) layer on the conductive glass

1. Identify the conducting side of a piece of conductive glass by using a multi-meter to measure its resistance.
2. With the conducting side up, tape the glass on three sides using one thickness of tape. Wipe off any fingerprints or oils using wet tissue with ethanol. Blow to dry the glass.



3. Add one to two drops of titanium dioxide suspension on the conductive glass and quickly spread it using glass rod.



4. Dry the coated titanium dioxide layer at least 5 minutes. Carefully remove the tape without scratching the TiO<sub>2</sub> coating.



5. Heat the glass on a hotplate at 450°C in the fume hood for 20 minutes. [Note: This requires a plate that gets quite hot.] Cool the coated glass slowly by turning off the hotplate. Transfer the glass to the white tile using the forceps. Store the coated TiO<sub>2</sub> conductive glass for later use.



#### B. Preparation of iodide electrolyte solution (KI<sub>3</sub>)

Weigh 0.127 g of iodine and 0.83 g of potassium iodide into a beaker containing 10 mL of ethylene glycol. Stir the solution mixture using glass rod until the solids are completely dissolved.

## Experimental procedures

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

1. Get the prepared piece of coated  $\text{TiO}_2$  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  $\text{TiO}_2$  will change color as the dye is absorbed and complexed with the  $\text{Ti(IV)}$ .



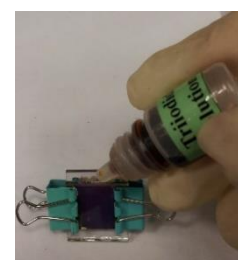
2. Rinse gently with water then with ethanol. Blow to dry the coated glass.
3. Pick another piece of glass to prepare the counter electrode. Identify the conducting side of the conductive glass first by using the multi-meter.
4. Wipe off any fingerprints or oils using wet tissue with ethanol and dry 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.
7. Measure and write down the  $V_{oc}$  (open circuit voltage) and  $I_{sc}$  (short circuit current) of the fabricated solar cell under room illumination and LED torch before adding electrolyte.

8. Add a few drops of a triiodide solution ( $\text{KI}_3$  solution) to the edge of the plate. Capillary action will cause the  $\text{KI}_3$  solution to fill up between the sandwiched plates.

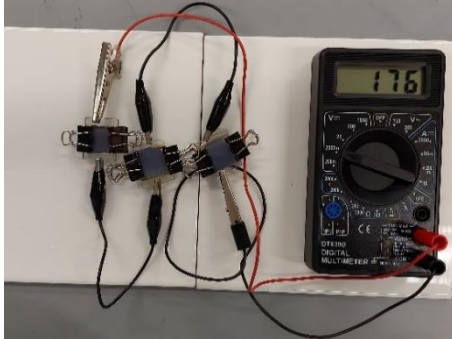


9. Connect the sandwich to the multi-meter using an alligator clip to each plate. [Hint: Do not scratch the surface of conductive glasses.]

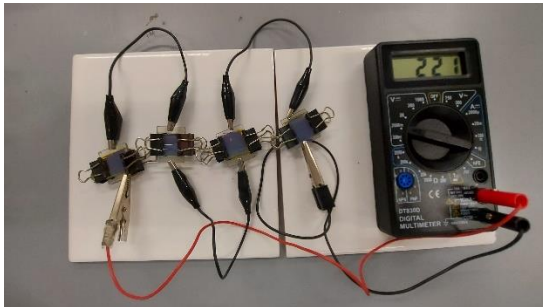
10. Measure and write down the  $V_{oc}$  (open circuit voltage) and  $I_{sc}$  (short circuit current) of the solar cell under room illumination, under LED torch, and in dark box.



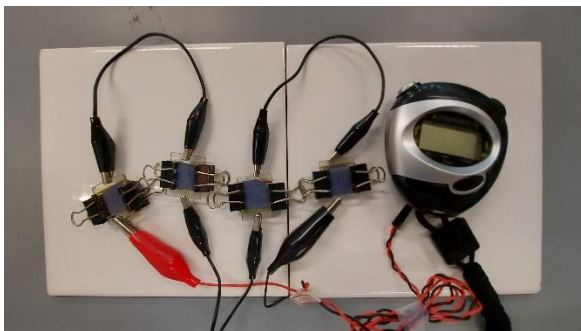
- B. Power on the stopwatch by connecting several pieces of DSSCs in series
1. Connect 3 pieces of DSSCs in series and measure the  $V_{oc}$  (open circuit voltage) and  $I_{sc}$  (short circuit current) of this system by multi-meter under illumination of LED torch.



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.

## Results and Discussion

### Data and data treatment

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

Type of Berries used: blueberries

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

|                   | $V_{oc}$ (open circuit voltage) |      | $I_{sc}$ (short circuit current) |            |
|-------------------|---------------------------------|------|----------------------------------|------------|
| Room Illumination | 0                               | (mV) | 0                                | ( $\mu$ A) |
| Under LED Torch   | 0                               | (mV) | 0                                | ( $\mu$ A) |

$\therefore$  Under no electrolyte conditions, the solar cell worked / did not work\*

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

|                   | $V_{oc}$ (open circuit voltage) |      | $I_{sc}$ (short circuit current) |            |
|-------------------|---------------------------------|------|----------------------------------|------------|
| Room Illumination | 90                              | (mV) | 0                                | ( $\mu$ A) |
| Under LED Torch   | 331                             | (mV) | 18                               | ( $\mu$ A) |
| In the dark box   | 3                               | (mV) | 0                                | ( $\mu$ A) |

$\therefore$  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 | $V_{oc}$ (open circuit voltage) |      | $I_{sc}$ (short circuit current) |            |
|-------------------|---------------------------------|------|----------------------------------|------------|
| 3                 | 970                             | (mV) | 11                               | ( $\mu$ A) |
| 4                 | 1320                            | (mV) | 11                               | ( $\mu$ A) |
| 5                 | 1550                            | (mV) | 11                               | ( $\mu$ A) |

No. of solar cells required to power on the stopwatch 4

Estimate the minimum voltage that can power on the stopwatch 1320 mV

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

\*Circle the appropriate answer in each question

Dye sensitized solar cell (DSSC) provides a technically and economically reliable alternative to the p–n junction photovoltaic devices. The function of dye molecules in DSSC is to absorb the incident light for photoexcitation of an electron to the higher energy state. To be an efficient photosensitizing agent, dye molecule must show excellent absorption in the visible region (400 nm to 700 nm), adsorb strongly on the surface of the semiconductor, have a high extinction coefficient and be stable in its oxidized form allowing it to be reduced by a redox electrolyte.

When incident photons on a DSSC are being absorbed by the photo-anodes, electrons are excited from the ground state to the excited state and subsequently transferred to the conduction band of the TiO<sub>2</sub>. This process oxidizes the photo-anodes causing the electrons to travel through the external wire to the cathode of the cell and returns back to the anode via the electrolyte. This reoccurring process of moving charges generates electricity.

Dye-sensitized solar cells are one of the most promising devices for solar energy conversion due to their reduced production cost, ease of production and low environmental impact, especially those sensitized by natural dyes.

The DSSC can generate a voltage upon light illumination. It gives higher voltage and current value under LED torch illumination when compared with those obtained in the dark box and under room illumination. A stopwatch can be powered on if the supply voltage is higher than 1200 mV. To generate enough voltage supply to power on the stopwatch, several pieces of DSSCs are connected together in series.

DSSC fabricated from the dye extracted from blueberry affords the highest device efficiency. It is due to the fact that the darker the color of the dye, the higher is the sunlight absorption which results in the enhanced photocurrent densities.

Although it may be easy to fabricate the DSSC, some attentions and precautions should be paid to ensure the device can function accordingly. The conductive glass used in this experiment is FTO (fluorine-doped tin oxide coated) glass. Only one side of the glass can conduct electricity and the other side is not. The functional materials must be sandwiched between the two conductive sides which can be identified by measuring the resistance with multi-meters. The blueberries TiO<sub>2</sub> coated electrode and the counter electrode must be blow-dried completely before “sandwiching” them together. The TiO<sub>2</sub> layer detaches easily from the conductive glass if electrodes are rubbed each other.

## Conclusion

Dye-sensitized solar cells (DSSCs) are based on the semiconductor, titanium dioxide nanoparticles which are coated with a light-absorbing dye and surrounded by electrolyte, and then sandwiched between an anode and a cathode. The light-absorbing dye extracted from blueberry is responsible for the conversion of light energy into electrical energy. The electrolyte solution contains iodide ions that facilitate the transfer of electrons to cathode. Energy harvested through this simple dye-sensitized cell can be used to drive an electrical system load.

In this experiment, students learn to fabricate a natural dye-based solar cells and measure the electrical properties of the cells. The hand-on laboratory activity includes (1) fabrication of the solar cell by coating the conductive glass of TiO<sub>2</sub> substrate with natural dye from blueberry; (2) determination of the electrical properties of the DSSCs under different conditions and; (3) application of DSSCs to power a stopwatch on.

## Questions and Answer

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?

*The voltage and the current generated by the solar cell increase with an increase in the light intensity shone on the solar cell.*

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

*Ethanol serves to remove water from the porous TiO<sub>2</sub> because ethanol is more volatile than water.*

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

*It is inexpensive, environment-friendly, able to be transparent, easy to process and realize to various colors; therefore, solar window and shingles are prospective applications in building integrated photovoltaics (BIPV). The availability of lightweight, flexible dye sensitized cells or modules are attractive for applications in room or outdoor light powered calculators, gadgets, and mobiles.*

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?

*At low temperatures, the electrolyte would freeze leading to a physical damage. High temperatures cause the liquid electrolyte to expand, making leakage of the electrolyte a serious problem.*

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