“Teaching Kit on Chemical Testing for Senior Secondary Curriculum”

Student Laboratory Manual

Department of Chemistry
Hong Kong Baptist University

A Project commissioned by
the Innovation and Technology Commission and
Hong Kong Council for Testing and Certification

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Important Note

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

Experiment 1 – Determination of NO_2 in Air: Air Pollution Analysis

Introduction

The air pollutant examined in this experiment is nitrogen dioxide, NO_2. NO_2 is one of the precursors in photochemical smog formation. In the presence of sunlight, NO_2 dissociates to form highly reactive atomic oxygen. The atomic oxygen released further initiates a series of chain reactions with hydrocarbons (i.e., volatile organic compounds, VOCs), nitrogen oxides (usually in the presence of light) to form compounds such as ozone and other oxidants including aldehydes, peroxyacetylnitrate (PAN). Aldehydes are toxic and can condense to form aerosols which limit atmospheric visibility. Ozone and PAN are not only hazardous to human but also extremely toxic to plants and can cause oxidative damage to many materials such as fabrics, plastics and rubber. In addition, they are also very powerful lachrymators or eye irritants.

To analyze airborne substances, the initial step is to sample them from the atmosphere. This can be achieved by using either the active or passive sampling techniques. A rather expensive constant flow rate pump is required in the active sampling process to pull airborne materials into an absorbent device. On the other hand, passive samplers are the preferred sampling devices for taking airborne molecules for subsequent instrumental analysis. Commercial passive sampling devices are available in the market. In this laboratory activity, a passive sampler covered by a Chinese Patent is used[1]. The passive sampler (Fig. 1) consists of a glass vial, a Teflon membrane and a screw cap which can hold a fixed amount of liquid absorbent. It allows natural diffusion of gas into the liquid. It is simple and easy to fabricate. They can be used to monitor simultaneously a large number of sampling sites. The sampling rate can be determined experimentally by the direct active sampling method and it is normally provided by the supplier.

NO_2 on the other hand can be generated indoors from the flue gas of burning stove and tobacco smoking. Thus, it has been identified as one of the indoor pollutants. High concentration of NO_2 in the indoor environment may cause eye, nose and throat irritation and could also impair lung functions and increase the risk of respiratory infections. For promoting health, the Environmental Protection Department (EPD) has launched an Indoor Air Quality Certification Scheme (www.iaq.gov.hk). Through the Hong Kong Laboratory Accreditation Scheme (HOKLAS)[2], a number of local commercial testing laboratories have been accredited for measuring the indoor NO_2 level. Most accredited laboratories are testing indoor air samples collected by the passive sampling method. NO_2 trapped in the sampler will be extracted by water and converted to nitrite ion for quantification with different instrumental analytical methods such as “flow injection method” or “ion chromatographic method”. However, the instruments used in the commercial laboratories are not available in secondary schools. On the basis of a literature procedure, a colorimetric method for the determination of NO_2 is used in both the indoor and outdoor environments.

In this laboratory work, NO_2 in air (either indoor or outdoor environment) is collected by a passive sampler. When NO_2 and the colourless absorbent reagent are brought together, a pink-colour solution is developed. The colour is due to the formation of an azo dye complex. Fig. 2 shows the absorption spectrum of the azo dye. Since the complex is the only coloured species in the system, the concentration of NO_2 can be determined by colorimetry. The absorbance is directly proportional to the concentration of the coloured constituent as governed by the Beer’s Law [3].

Recently, a very simple protocol for teaching colourimetry using smartphone was described[4]. To exploit modern information technology, the Chemistry Department of

Hong Kong Baptist University (HKBU) has developed a mobile app “ChemEye” as one of the new options for detecting colour species. The application of “ChemEye” in the detection of NO_2 in air will be demonstrated in this experiment. The in-app calculation and conversion have simplified the steps of manual calculation and graph sketching, enriching the learning experience by this simple, handy and convenient method.

Fig. 1. Passive samplers.

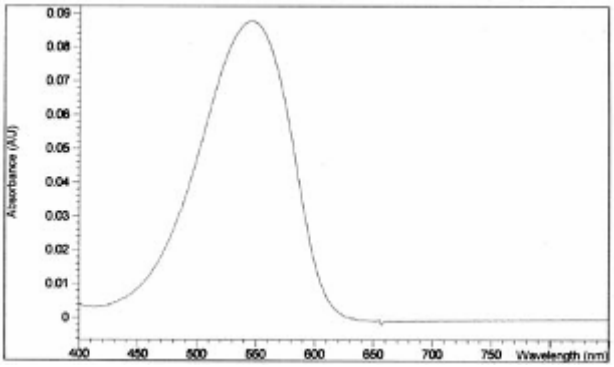


Fig. 2. Visible absorption spectrum of the azo dye.

Intended Learning Outcomes

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

1. understand the operation of the testing and certification sector in Hong Kong;
2. isolate NO_2 in the air samples using passive sampling techniques;
3. analyze NO_2 quantitatively using colorimetry;
4. acquire the basic concepts of accuracy, precision, and detection limit of analytical methods;
5. acquire the knowledge and technique of using a mobile device installed with “ChemEye” to determine NO_2 in air.

Experimental

Apparatus

- 1x 4-LED photometer and/or mobile device installed with “ChemEye”
- 2x passive sampler with stand
- 1x membrane
- 1x glass cuvette (for 4-LED photometer detection)
- 9x glass test tubes (for ChemEye detection)
- 1x test tube rack (for ChemEye detection)
- 4x volumetric flask of 25 mL
- 1x pipette of 5 mL
- 1x 100 – 1000 μL auto-pipette with pipette tubes or 1 mL graduated pipette
- 1x 50 – 200 μL auto-pipette with pipette tubes or 0.2 mL graduated pipette
- 1x scissors
- 1x beaker of 100 mL
- 1x beaker of 250 mL
- 2x dropper

QR codes for downloading “ChemEye” in iOS (left) & Android (right)

Reagents and chemicals

- 2.5 g sulphanilic acid
- 0.025 g *N*-(1-naphthyl)-ethylenediamine dichlorohydrate
- 5 mL 1-propanol
- 0.75 g sodium nitrite
- 2 L Deionized water

Sample pretreatment method

1. Set-up of gas samplers

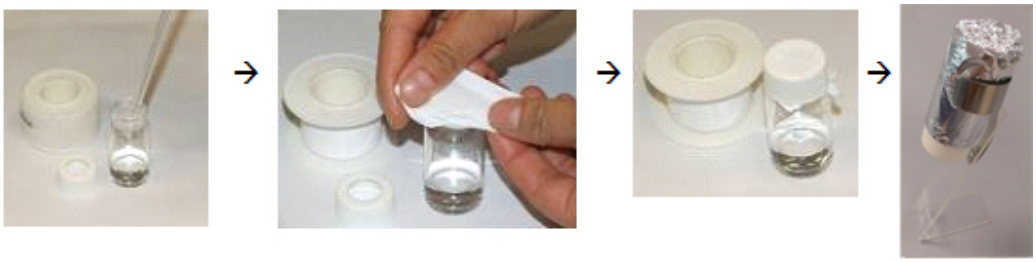
   i. Pipet 5 mL of absorbent reagent into the sampling vial (i.e., sampler).

   Absorbent reagent: ***A*** *- Dissolve 2.5 g of sulphanilic acid,* ***B*** *- 0.025 g of N-(1-naphthyl)-ethylenediamine dichlorohydrate, 5 mL of 1-propanol, and make up to 500 mL with deionized water.*

   ii. Mount a membrane onto the opening of the vial.

   iii. Screw the hole-cap to fix the membrane in position.

   iv. Wrap the vial with aluminum foil. Invert the sampler and let it stand near the roadside for 1.5 hours.



   v. When time is up, replace the membrane cover with a septum cap and let it stand for 10 min.

   vi. Repeat the above set-up steps on the other two sample vials and use the vial with a solid cap as control.

2. Preparation of a series of NO_2 standard solution in 25 mL volumetric flask as follows:

Standard Solution	**1**	**2**	**3**	**4**
Concentration of NO_2 (ppm)	0.02	0.04	0.08	0.12
Volume of 10 ppm NO_2 added (mL)	0.05	0.1	0.2	0.3

Then all the solutions are filled up to the mark with the *absorbent reagent*.

10 ppm NO_2: Dilute 5 mL of 1000 mg/L (ppm) NO_2 solution to 500 mL in a volumetric flask with deionized water.

1000 ppm NO_2: Dissolve 0.7499 g $NaNO_2$ in a 500 mL volumetric flask and dilute to the mark with deionized water.

Analytical method

Let the colour develop for 10 min and then measure the absorbance of the blank (absorbent reagent), sample and standard solutions using the photometer with a green LED light source and/or mobile device installed with "ChemEye".

Safety precaution

- Observe the standard safety procedures for laboratory activity;
- Put on the safety goggles, laboratory coats, and gloves;
- Be careful when cutting the membrane with a pair of scissors;
- Material Safety Data Sheet (MSDS) of chemicals are available online on the website of MSDSonline.com.

Data Sheet for 4-LED photometer

Analysis of NO_2 content near road side

A = Absorbance, E_o = Detected potential of the blank $\quad \therefore A = \log\left(\frac{E_o}{E}\right)$

Absorbance = $\log\frac{E_o}{E}$ using **green** LED measurement, E_o = _____V

Sampling time: _____min

Sampling location: _____

Sampling rate (provided): 2.3 x 10^{-5} m^3/min

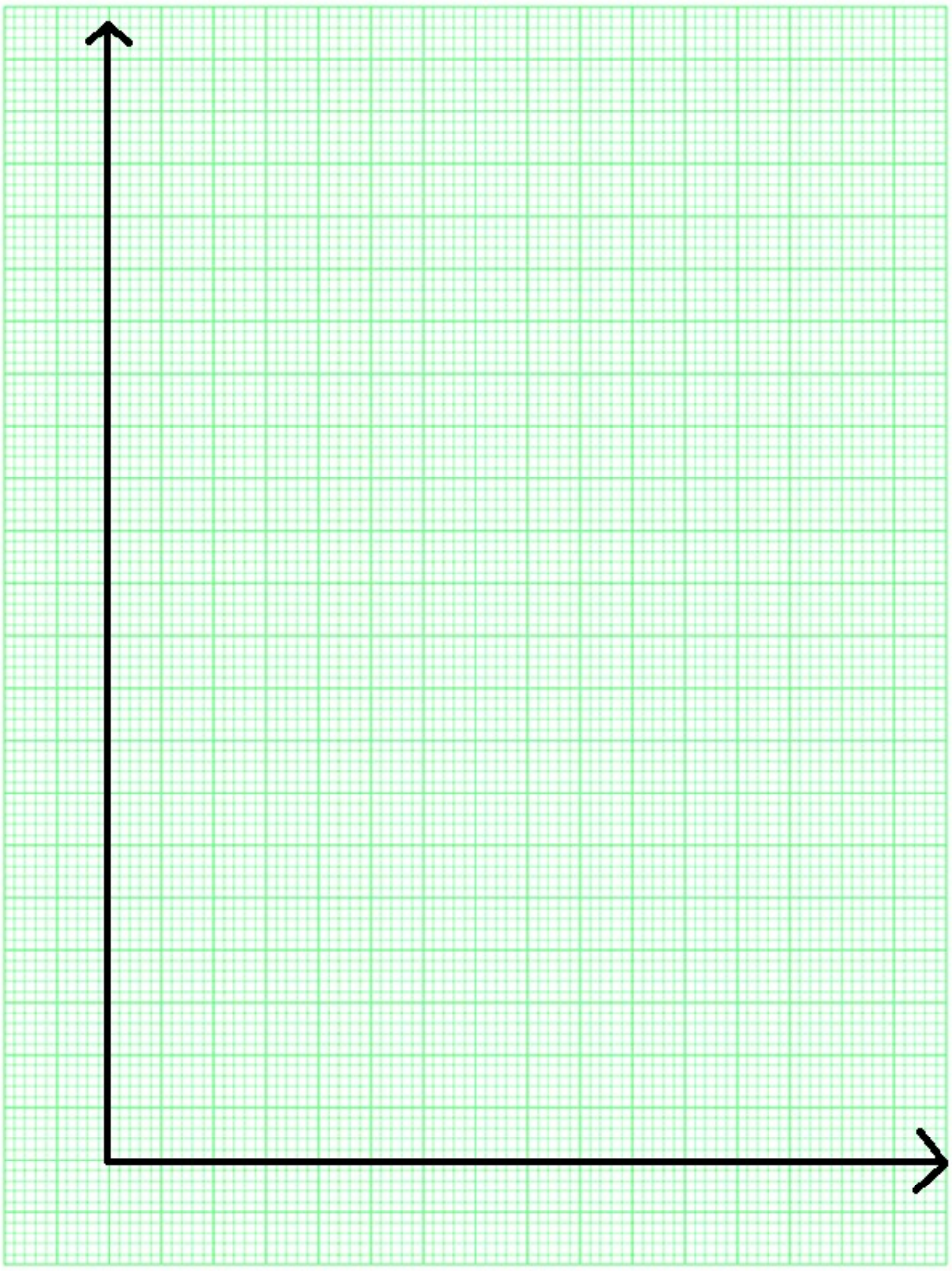
Descriptions	E (V)
Blank	E_o =
0.02 ppm NO_2 standard	
0.04 ppm NO_2 standard	
0.08 ppm NO_2 standard	
0.12 ppm NO_2 standard	
Sample 1st trial	
Sample 2nd trial	
Sample 3rd trial	
Control	

Data Treatment for 4-LED photometer

1. Calculate the absorbance (A) for the standards, sample solutions and control.

Descriptions	E (V)	Absorbance
Blank	E_o =	0
0.02 ppm NO_2 standard		
0.04 ppm NO_2 standard		
0.08 ppm NO_2 standard		
0.12 ppm NO_2 standard		
Sample 1st trial		
Sample 2nd trial		
Sample 3rd trial		
Sample Mean	----------	
Control		

2. Plot a calibration curve {absorbance vs. concentration of NO_2 (ppm)} and find out the concentration of NO_2 (mg/L) in the samplers and the control.



The concentration of NO_2 (mg/L) in the sampler: _____

The concentration of NO_2 (mg/L) in the control: _____

Data Sheet for ChemEye

I. Calibration Curve

1. Which curve have you chosen for the calibration? R, G or B value?

2. Please state the reason(s) of choosing the respective graph as the calibration curve?

II. Analysis of NO_2 content near road side

Sampling time: _____min

Sampling location: _____

Sampling rate (provided): 2.3×10^{-5} m^3/min

Descriptions	____-value	Concentration
Sample 1st trial		
Sample 2nd trial		
Sample 3rd trial		
Sample Mean	----------	
Control		

The concentration of NO_2 (mg/L) in the control:_____

Calculation of NO_2 content in atmosphere

Use the treated data set either from 4-LED photometer or ChemEye as detection method, calculate the NO_2 content in the atmosphere.

1. Calculate the weight of NO_2 collected in the samplers and the control:

   Weight of NO_2 in sampler (μg):

   Conc. of NO_2 (mg/L) in sampler $\times$ 10^3 μg/mg $\times$ 5 mL $\times$ 10^{-3} L/mL

   Weight of NO_2 in control (μg):
   Conc. of NO_2 (mg/L) in control $\times$ 10^3 μg/mg $\times$ 5 mL $\times$ 10^{-3} L/mL

   Net weight of NO_2 collected (μg):
   Weight of NO_2 in sampler (μg) - Weight of NO_2 in control (μg)

2. Calculate the volume of air sample (m^3):
   Volume of air sample (m^3): Diffusion rate (m^3/min) x sampling time (min)
   = 2.3 x 10^{-5} m^3/min x sampling time (min)

3. Calculate the concentration of NO_2 in atmosphere with unit μg/m^3.

$$NO_2\ (\mu\ g/\ m^3) = \frac{\text{Weight of } NO_2\ (\mu\ g)}{\text{Volume of Air Sampled in } m^3}$$

Questions

1. What is the colour change of absorbent reagent after standing near roadside for 1.5 hours?

2. Green light source is used for taking measurements in the experiment. Suggest the reasons.

3. What are the major differences between passive and active samplers for NO_2 analysis?

4. Why must the sampler be wrapped with aluminum foil?

5. Find out about the “flow injection method” or “ion chromatographic method” that the testing and certification sector uses to measure NO_2 in IAQ. What are the advantages of using these methods?

References

[1] 陳永康,肖丹,王柯敏,<快速簡便氣體採集和測定裝置> 中國專利<ZL 00 2 25023.3>, 22/04/2001. The Intellectual Property holder (i.e., Wing Hong Chan) of this Patent agrees to permit the use of the Patent for education purpose in Hong Kong.

[2] http://www.hkas.gov.hk/

[3] D. Xiao, L. Lin, H. Yuan, M. F. Choi, W. H. Chan, *J. Chem. Educ.* **2005**, *82*, 1231-1233.

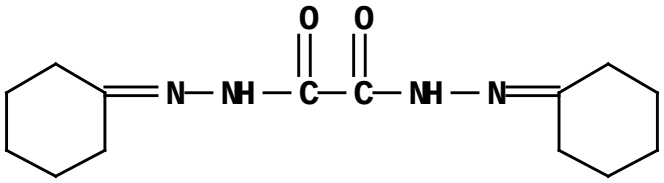
[4] T. S. Kuntzleman, E. C. Jacobson, *J. Chem. Educ.* **2016**, *93*, 1249-1252.

Experiment 2 – Analysis of Copper in Wastewater

Introduction

Industrial effluents and wastewater may pose potential risks and hazards to human beings and the environment. In general, effluents from electroplating plants may contain toxic metal ions such as copper (II) ions. Repeated or prolonged exposure to Cu (II) species such as copper sulfate can cause kidney and liver damage[1]. To comply with the local legislation, companies can engage testing laboratories in Hong Kong which are capable of analysing the copper content to ensure that the effluents do not exceed the upper limit of 0.2 mg/L[2] before discharging into the environment. The concentration of Cu (II) in industrial effluents can be determined by "colorimetric" method.

Cu (II) reacts with oxalic acid bis(cyclohexylidene hydrazide) (cuprizone) to form a complex with a broad band absorption in the visible light range (Fig. 1)[3]. The absorbance of this complex is insensitive to pH change and is therefore commonly used for the determination of copper. Classically, the concentration of Cu (II) can be determined by comparing visibly the intensity of the orange colouration with Cu (II) standards. For a more accurate quantitative result, colorimetric method can be used.



Oxalic acid bis(cyclohexylidenehydrazide) – (Cuprizone)

The deepness of the colour, usually measured as the absorbance (A) of the solution containing the absorbing analyte, is proportional to the extent of the absorption of

characteristic light by the coloured compound. The absorbance of a coloured species can be correlated with the concentration of the species according to the Beer's Law, which states that: $A = \varepsilon bc$ where A is the absorbance, ε is the molar extinction coefficient, c is the concentration of the species and b is the path length of the optical cuvette. The absorbance of a solution is defined by $A = \log(I_o/I)$ where I_o and I are the initial and final light intensity detected after passing through the analyte solution, respectively. The deepness of the colour of the copper-cuprizone complex is proportional to the copper contents. The absorbance of the analyte solution will be measured by a colorimeter or a spectrophotometer and compared with those obtained from standard copper solutions.

In this experiment, a yellow Light Emitting Diode (LED) is used as the radiation source (Fig. 2). The radiation after passing through the absorbing analyte is allowed to fall on a photo-transistor which converts light energy into an electric signal. The signal is proportional to the irradiation intensity and can be amplified and measured.

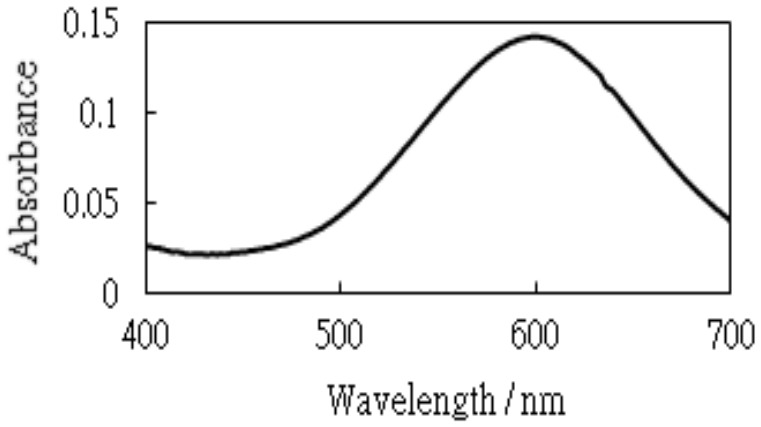


Fig. 1. Visible spectrum of Cu (II)-Cuprizone.

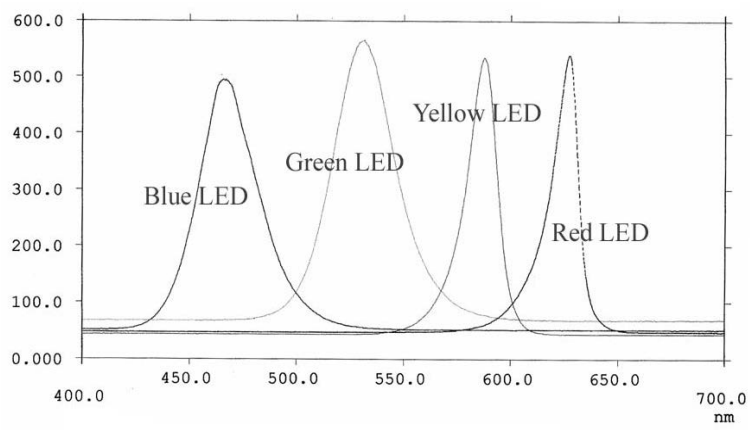


Fig. 2. Emission spectrum of four coloured LED.

Intended Learning Outcomes

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

1. understand the operation of the testing and certification sector in Hong Kong;
2. analyze Cu (II) quantitatively using colorimetry;
3. acquire the basic concepts of accuracy, precision, and detection limit of analytical methods;
4. acquire the knowledge and technique of using a mobile device installed with "ChemEye" to determine Cu(II) content in the wastewater.

Experimental

Apparatus

- 2x Spatula
- 4x beaker of 100 mL
- 1x Pyrex bottle of 100 mL
- 1x volumetric flask of 250 mL
- 7x volumetric flask of 25 mL
- 2x dropper
- 2x rubber teats

- 1x 100 – 1000 μL auto-pipette with pipette tubes or 1 mL graduated pipette
- 1x hot plate
- 1x 4-LED photometer or mobile device installed with "ChemEye"
- 1x plastic cuvette (for 4-LED photometer detection)
- 6x glass test tubes (for ChemEye detection)
- 1x test tube rack (for ChemEye detection)

QR codes for downloading "ChemEye" in iOS (left) & Android (right)

Reagent and chemicals

- Oxalic acid bis(cyclohexylidene hydrazide)
- Copper (II) sulfate
- Ethanol
- Citric Acid
- 25 % Ammonia Solution

Laboratory preparation

1. Preparation of cuprizone reagent

   Dissolve 0.5 g oxalic acid bis(cyclohexylidene hydrazide) in 100 mL 50% ethanol with heating. The reagent solution is stable for about three months if stored in well-closed containers in a cool place.

2. Preparation of citrate buffer

   Dissolve 37 g citric acid to 100 mL deionized (D.I.) water in a 250 mL beaker. Treat the solution with 95 mL 25% ammonia solution with stirring, let the solution cool to room temperature, and transfer the resulting solution to a 250

mL volumetric flask and make up to the mark with D.I. water.

3. Preparation of 100 ppm of copper standard solution

   Dissolve 0.251 g copper (II) sulfate in 25 mL D.I. water. Transfer the resulting solution to a 100 mL volumetric flask and make up to the mark with D.I. water. This stock solution will have a copper concentration of 1000 ppm. Transfer 2.5 mL 1000 ppm of copper standard solution in a 25 mL volumetric flask and add D.I. water to the mark.

Analytical method

1. Measurement of Cu concentration by Cu (II)-Cuprizone complex

   i. Preparation of standard Cu (II) solutions and unknown solution

   Prepare a series of Cu (II)-Cuprizone standard solutions by mixing different amounts of 100 ppm Cu (II) stock solution and citrate buffer, then followed by adding 1.00 mL cuprizone according to the table below^:

	Volume of 100 ppm Cu (II) stock solution (mL)	Volume of Cuprizone (mL)	Volume of citrate buffer (mL)	Final concentration (ppm)	Final volume (mL)
Standard #1	0.10	1.00	2.5	0.40	25.0
Standard #2	0.20	1.00	2.5	0.80	25.0
Standard #3	0.30	1.00	2.5	1.20	25.0
Standard #4	0.40	1.00	2.5	1.60	25.0
Standard #5	0.50	1.00	2.5	2.00	25.0
Blank	0.00	1.00	2.5	0.00	25.0
Unknown	*	1.00	2.5	--	25.0

* Pipette 1.00 mL unknown solution to a 25 mL volumetric flask.

^ For 4-LED photometer detection: Standard #1 to #5, blank solution and sample

^ For ChemEye detection: Standard #2 to #5, blank solution and sample.




2. Spectrophotometric measurements

4-LED photometer

I. Procedure

i. Turn on the power of the colorimeter, turn on the Yellow LED and allow 15 minutes to stabilize.

ii. Click to the “Calib” position

iii. Adjust the “Light Level” knob until the LCD display reads about 4.50 V.

iv. Fill the sample tube with the reagent blank.

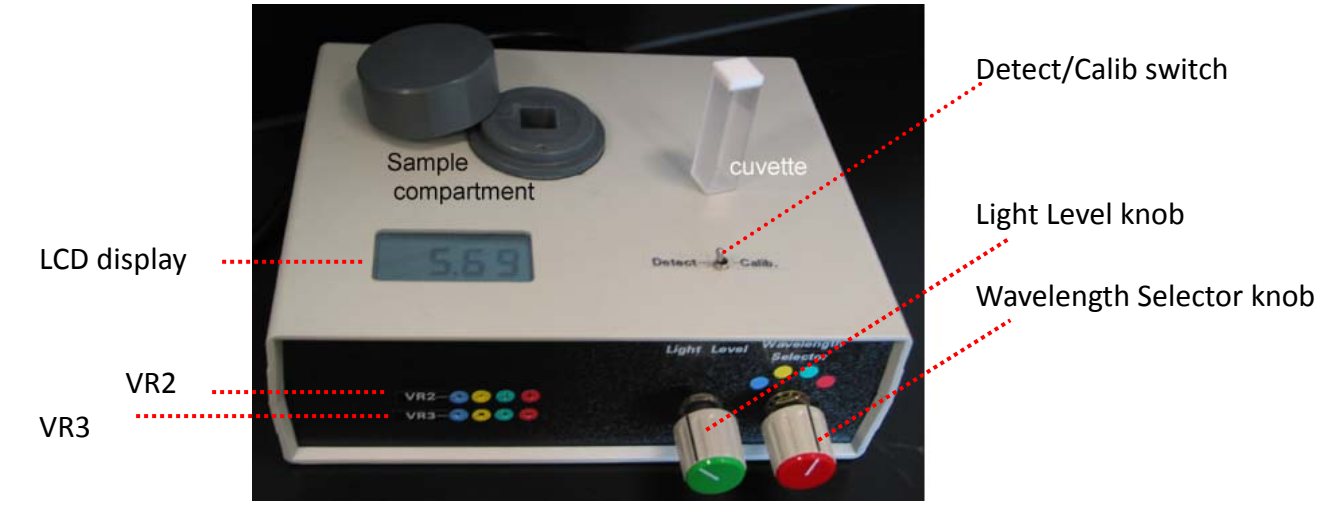
v. Remove the cover of cell holder.

vi. Click to the “Detect” and measure the blank sample by inserting the blank into the cell compartment.

vii. Record the reading (E_o) shown on the LCD display.

viii. Rinse the cuvette and then fill it with the standard solution and record the reading (E) again.

ix. Repeat the procedure with series of standard solutions and sample solution. (Caution: Do not adjust the “Light Level” knob while taking the standard and sample measurements)



II. Data treatment

Plot a calibration graph of the standards using $A = \log\left(\frac{E_o}{E}\right)$. Determine the concentration of the sample solution from the calibration curve using

$$A_{sample} = \log\left(\frac{E_o}{E_{sample}}\right)$$

$$A = \log\left(\frac{E_o - E_\infty}{E - E_\infty}\right)$$

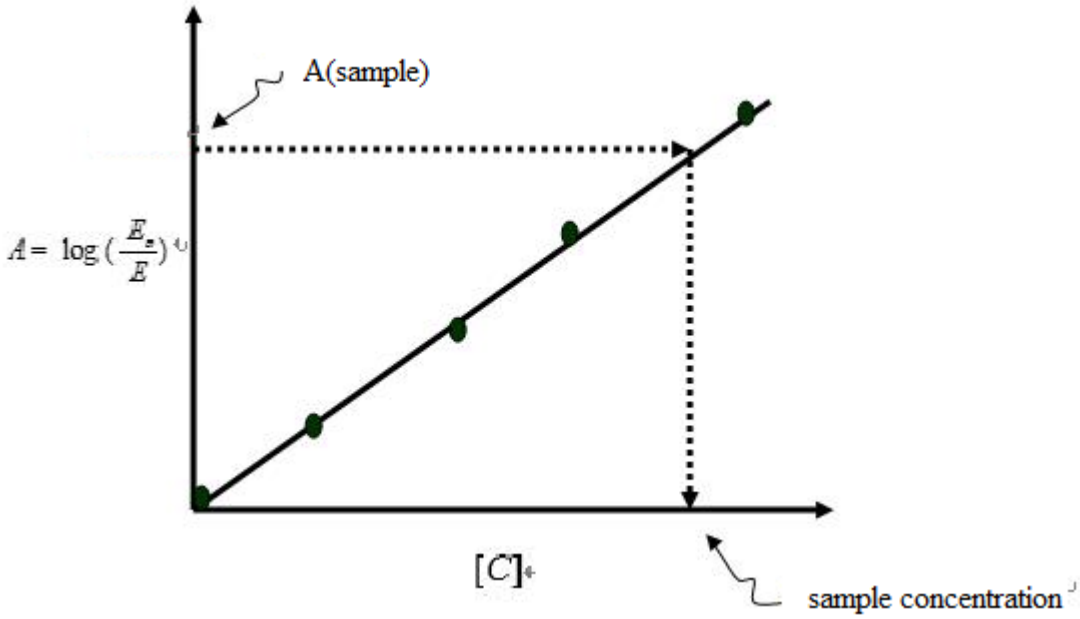
where E is the potential measured with the sample placed in the sample holder,

A is Absorbance

E_o is the potential measured with the blank solution, and

E_∞ is the potential in the absence of light (dark current) (assuming = 0 V)

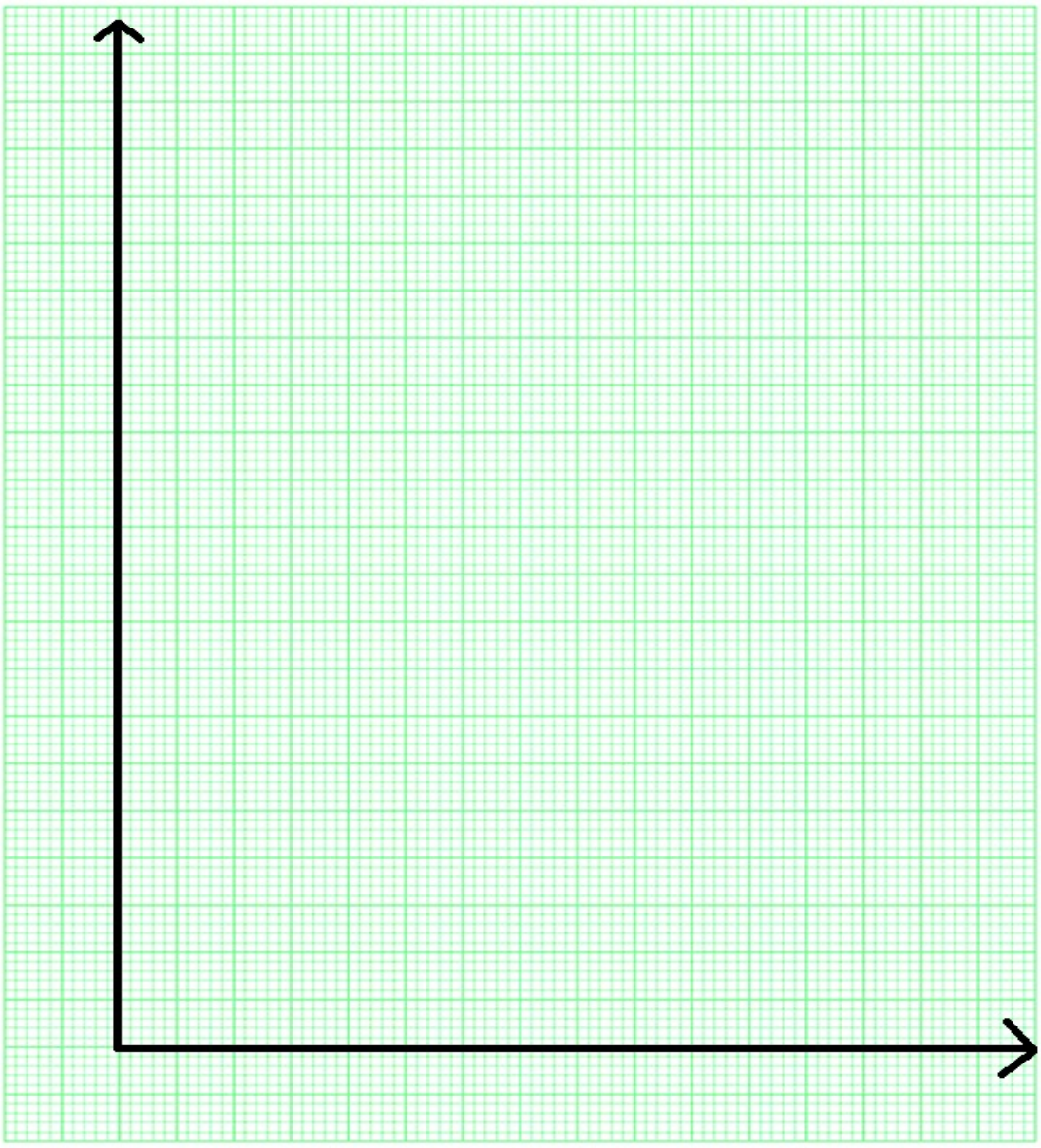
$$\therefore A = \log\left(\frac{E_o}{E}\right)$$



Data Sheet for 4-LED Photometer (Yellow LED)

Calibration Voltage =			
	(E)	$\log\left(\frac{E_\circ}{E}\right)$	[Cu] /ppm
Blank	E_o =_____	0.000	0.000
Standard #1			
Standard #2			
Standard #3			
Standard #4			
Standard #5			
Sample			
Linear coefficient (R^2) =			

Plot a calibration curve {absorbance vs. concentration of copper (ppm)} and find out the concentration of copper (ppnm) in the sample.



ChemEye

I. Procedure

i. Install “ChemEye” from AppStore or PlayStore.

ii. Open the app. Press “Start” and create a new absorbance profile.

iii. Enter the name of the profile. (e.g. Copper)

iv. Choose the appropriate unit for the detection. For this experiment, the unit

is part per million (ppm). Enter the number of data in the calibration curve (from 2 to 5 points). In this experiment, 5 data points are required for plotting the calibration curve.

v. After entering all information required, camera of the mobile device is turned on for the detection of blank, standard solutions and sample.

vi. Place the green square shown in the detection page on the blank/standard solution. Press" get color" to capture the RGB value of the solution. After that, enter the respective concentration.

vii. Repeat the step above for the sequence from the lowest concentration to the highest (i.e.:0 ppm, 0.80 ppm, 1.20 ppm, 1.60 ppm and 2.00 ppm).

viii. Input all the information required for constructing the calibration curve, press "Finish and create absorbance profile".

ix. Three calibration curves will be generated from converting the RGB value captured to absorbance. From the FAQ section, there are some guidelines for selecting which curve best fit to the detection of analyte.

x. Select the best curve and press "save". Sample can be detected immediately right after saving the curve. Detect the sample solution by pressing "get colour" in the detection page.

xi. The concentration of detected sample is calculated by the app and shown on the screen.

II. Data and data treatment

The RGB value captured from the colour in the image of the standard solution is converted to absorbance by the app. After that, the app will generate three respective calibration curves, showing the relationship of absorbance against the copper concentration in standard solutions. The linear equation and linear

coefficient are also shown in the selection page. After saving the curve, sample detection can be proceeded. The concentration in the sample solution is then calculated by the pre-saved linear equation.

Datasheet for ChemEye

1. Which curve have you chosen for the calibration? R, G or B value?

2. Please state the reason(s) of choosing the respective graph as the calibration curve?

3. What is the slope and linear coefficient (R^2) of the calibration curve selected?

4. What is the concentration of copper in the wastewater?

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 chemicals are available online on the website of MSDSonline.com

Questions

1. In the electroplating industry, metals other than copper may be released into the surrounding water. State the names of the metals.

2. Cuprizone is used to form complex with Cu^{2+} before measurement. Do you think that Cu^{2+} can be determined directly without addition of cuprizone? Why?

3. Will the calibration curve change if a Green LED (wavelength of roughly 520–570 nm) is used instead of the yellow one?

References

[1] Canadian Centre for Occupational Health and Safety, *Material Safety Data Sheets: Copper Sulfate Pentahydrate*, Hamilton, 2013.

[2] Environmental Protection Department, HKSAR, *Water Pollution Control Ordinance, Chapter 358 Proposed Amendments to the Technical Memorandum on Effluent Standards*, HKSAR, 2001.

[3] E. B. Sandekk, *Photometric Determination of Traces Metals*, 4th edition; Wiley: New York, 1989.

Experiment 3 – Determination of Sulphur Dioxide (SO_2) in Dried Food Using Optimized Monier-Williams Method

Introduction

Fresh and raw food are limited in supply. Moreover, food is not always consumed immediately after harvest or slaughter. Food has to be transported from where it is produced to the consumer. Food spoilage and corresponding prevention is, therefore, a global concern of human health and economy. Food scientists and technologists are working hard to find ways to preserve food, so that the food can be stored and transported without deterioration in quality over a period of time. There are many preservation techniques, such as heat treatment, irradiation, drying, chilling or freezing, sugaring, and salting. The use of chemical preservatives is so far the most common method adopted in the food industry. The basic principle of using chemical preservatives is to kill microorganisms and to inhibit microbial growth on food items. One of the common chemical preservatives is sulphur dioxide. Sulphur dioxide is commonly used to dehydrate and preserve food items because it can also prevent browning of food and help to preserve the natural colour and flavor during the drying process. Trace amount of sulphur dioxide is, therefore, found in dried fruits and vegetables; and the fruit juices and alcoholic beverages made from dried fruits.

Sulphur dioxide (SO_2) is a gas produced by the combustion of elemental sulphur. The gas gives an unpleasant smell similar to rotten eggs. The gas is water soluble resulting in sulfurous acid (H_2SO_3). Exposure to high level of sulphur dioxide through inhalation and ingestion can cause breathing problems, emphysema, and chronic bronchitis over time. Although the amount of sulphur dioxide in food is not high enough to give rise to any of these respiratory diseases, individuals who are

hypersensitive to sulphur dioxide may have allergic problems after ingestion. Symptoms include shortness of breath, headache, and nausea. Serious allergic reactions may even result in death. It is, therefore, necessary to determine the amount of sulphur dioxide in food.

The determination of sulphur dioxide content is one of the important aspects in food analysis of the testing and certification sector. Standard methods of AOAC Official Method 990.28 and 990.29 are widely used in local commercial testing laboratories. According to the Food and Drugs (Composition and Labelling) Regulations of the Laws of Hong Kong (Cap 132W), the functional class of sulphur dioxide and the corresponding name shall be specified in the list of ingredients if a food consists of or contains sulphur dioxide in a concentration of 10 mg/kg or more. The public, who are concerned with potential health risks associated with sulphur dioxide in food, should read the food label carefully prior to consumption of the food.

In this experiment, the optimized Monier-Williams method will be used for quantitative determination of the sulphur dioxide content in dried food. The determination follows the standard method of AOAC Official Method 990.28. Dried food is purchased from the local market and is used as real-life sample. The sample is heated with reflux in a water-ethanol mixture. A stream of air is used to sweep sulphur dioxide through a condenser via bubbler to the receiver with hydrogen peroxide solution (H_2O_2), where sulphur dioxide is oxidized to sulfuric acid. The amount of sulfuric acid produced is directly proportional to the sulphur dioxide content in the sample and is determined by titration with pre-standardized sodium hydroxide solution (NaOH). The principle and the content of this experiment could confer students with the importance of analytical technique of acid-base titration and

knowledge of sampling and quantifying the sulfur dioxide in real-life sample of dried food using appropriate tests.

Intended Learning Outcomes

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

1. understand the operation of the testing and certification sector in Hong Kong;
2. isolate sulphur dioxide from dried food using reflux distillation;
3. analyze sulphur dioxide quantitatively using acid-base titration method;
4. acquire the basic concepts of accuracy and precision of analytical methods.

Experimental

Apparatus

- 1x two necked round bottom flask of 500 mL
- 1x water condenser
- 1x still head
- 1x screw adapter
- 3x dropper
- 3x 1 meter long rubber tubing
- 1x air pump
- 1x heater
- 4x conical flask of 250 mL
- 2x beaker of 250 mL
- 1x measuring cylinder of 100 mL
- 1x burette of 50 mL
- 1x scissors

Fig. 1. Glass apparatus and air pump.

Fig. 2. Gas collecting tube.

Reagents and chemicals

- 20 g dried food sample
- 250 mL water-ethanol solution (95:5 v/v)
- 50 mL 3% hydrogen peroxide
- 250 mL 0.01 M sodium hydroxide solution
- 50 mL 6 M hydrochloric acid
- 2 g potassium hydrogen phthalate
- 10 mL methyl red
- 10 mL phenolphthalein
- 1 L deionized water

Fig. 3. Reagents required.

Sample pretreatment method

1. Scissor the dried food sample into small pieces and weigh approximately 12.5 g sample into a two necked round bottom flask of 500 mL. (Fig. 4).
2. Add 200 mL water-ethanol mixture (95:5 v/v) to the round bottomed flask.
3. Add 30 mL 3% hydrogen peroxide solution to a conical flask of 250 mL.
4. Add a few drops of methyl red to the 3% hydrogen peroxide solution.
5. Then, add a few drops of 0.01 M sodium hydroxide solution until the colour of the solution turns yellow.

Fig. 4. Sample in the two necked round bottom flask.

6. Assemble the setup as shown in Fig. 5.
7. Disconnect the air purge stopcock and add 30 mL 6 M hydrochloric acid to the round bottom flask and then connect the air purge immediately.
8. Heat the solution for 60 minutes.

Fig. 5. Experimental setup

Analytical method

Standardization of sodium hydroxide solution

1. Weigh approximately 0.05 g potassium hydrogen phthalate into a conical flask of 250 mL.
2. Dissolve the potassium hydrogen phthalate using 50 mL deionized water and add several drops of phenolphthalein.
3. Titrate the solution with 0.01 M sodium hydroxide solution until the solution changes to permanent faint pink colour.
4. Record the volume of sodium hydroxide solution used and calculate the real concentration of the sodium hydroxide solution (standardization).
5. Repeat the steps of 1 – 4 two times more in order to obtain the mean and standard deviation of the real concentration of sodium hydroxide.

Determination of sulphur dioxide content by titration

1. After heating of 60 minutes, titrate the 3% hydrogen peroxide solution with the standardized sodium hydroxide solution until the solution changes to yellow colour.
2. Record the volume of sodium hydroxide solution used and calculate the sulphur dioxide content (mg/kg) in the dried food sample.

Safety precaution

- Observe the standard safety procedures for laboratory activity
- Put on the safety goggles, laboratory coats, and gloves
- Be careful when handling corrosive chemicals, such as concentrated acids
- The experiment can be carried out in a fumehood to avoid inhalation of vapours
- Material Safety Data Sheet (MSDS) of chemicals are available online on the website of MSDSonline.com

Data Sheet

Sample pretreatment method

Observations:

Analytical method

Standardization of sodium hydroxide solution

Observations:

	1st trial	**2nd trial**	**3rd trial**
Mass of KHP (g)			
Final volume (mL)			
Initial volume (mL)			
Volume of NaOH used (mL)			

Chemical equation involved:

$$NaOH\ (aq) + KHP\ (aq) \rightarrow Na^{+}\ (aq) + KP^{-}\ (aq) + H_2O\ (l)$$

	1st trial	2nd trial	3rd trial
Number of mole of KHP (mol)			
Number of mole of NaOH (mol)			
Concentration of NaOH (M)			

∴ The real concentration of the sodium hydroxide solution = _____ ± _____ M

Determination of sulphur dioxide content by titration

Observations:

Mass of dried food sample (g)	
Final volume (mL)	
Initial volume (mL)	
Volume of NaOH used (mL)	

Chemical equations involved:

$$H_2O_2\ (aq) + SO_2\ (g) \rightarrow H_2SO_4\ (aq)$$

$$2NaOH\ (aq) + H_2SO_4\ (aq) \rightarrow Na_2SO_4\ (aq) + 2H_2O\ (l)$$

Number of mole of NaOH (mol)	
Number of mole of SO_2 (mol)	
Mass of SO_2 (g)	
SO_2 content (mg/kg)	

∴ The sulphur dioxide content of = _____ mg/kg

Questions

1. Sulphur dioxide can act as a preservative in food, but the corresponding gas formed by itself is an air pollutant. Suggest an instrumentation method to monitor and measure the sulphur dioxide content in air.

2. In addition to sulphur dioxide, suggest another chemical that can act as a preservative in food and state the corresponding harmful effect to human.

3. Propose a method to reduce the amount of sulphur dioxide in food prior to consume.

4. Name some other chemical preservatives that the testing laboratories can test.

References

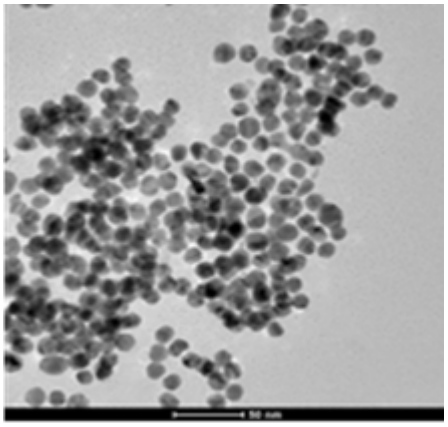
[1] AOAC International: *Official Methods of Analysis of AOAC* (2005), 18th Edition., ed. Horwitz W., Gaithersburg: MD, Method 990.28.

[2] AOAC International: *Official Methods of Analysis of AOAC* (2005), 18th Edition., ed. Horwitz W., Gaithersburg: MD, Method 990.29.

[3] *The European Standard EN 1988-1: 1998, Foodstuffs – Determination of Sulfite – Part 1: Optimized Monier-Williams method*, British Standards Institution, London.

Experiment 4 – Is This Dairy Product Safe? Gold Nanoparticles As A Visual Detection Tool of Melamine

Introduction



TEM (Transmission Electron Microscopy) image of 13 nm gold nanoparticles.

Nanotechnology is the utility of nano-sized materials. We can nowadays easily find the daily applications of nanotechnology in areas such as textiles, food packaging, and sewage treatment for their unique advantages. Many researches are being carried out for their potential uses in the biomedical and clinical field.

The chemical and physical properties of nanoparticles are very different from their respective elements in bulk. The physical properties of nanoparticles, in particular their optical (light absorption and emission) properties, are highly dependent of their chemical compositions, size, and shape. By controlling the reaction conditions, nanoparticles can be made into different sizes and morphology for their unique applications.

Food safety has been gaining substantial attention from the public for years including those products imported from the surrounding regions. One of the incidents was that melamine, a non-protein chemical rich in nitrogen, was illegally added into infant formula to increase its apparent protein content as the dairy industry normally checks the protein level through tests measuring nitrogen content e.g. the Kjeldahl method. Excessive intake of melamine causes adverse effects in babies including the formation of kidney stones. The identification and quantification of melamine then drew considerable attention in food industry. Dairy products, including pasteurized milk, formula milk, and chocolates, are monitored for melamine contamination in testing laboratories by chromatographic methods. The quantification of melamine can be

performed by liquid chromatography-mass spectrometry/mass spectrometry (LC-MS/MS) with the detection limit of several parts-per-million (ppm).

To suit the school environment, here we will adopt a simple and sensitive approach to detect melamine in milk samples using gold nanoparticles. In this experiment, you will learn about nanomaterial chemistry and food analysis. Gold nanoparticles of the size of 13 nm will be prepared by citrate reduction of Au (III) to Au (0). The as-prepared gold nanoparticle is well-dispersed in water and it gives a clear crimson colour solution. It will be used as a probe to detect the content of melamine in milk samples. Prior to the test, the milk samples will be pre-treated to remove the protein and fat contents. Students will thus learn about precipitation chemistry and will gain hands-on experience of solid-phase extraction, an important technique widely used in the testing and certification sector.

In the presence of melamine, the individual gold nanoparticles will be “cross-linked” by the melamine molecules via hydrogen bonding (each melamine molecule offers three sites of hydrogen bonding) to form a cluster. This results in an observable colour change from crimson to purple blue because the solution colour is nanoparticle-size dependent. The higher the content of melamine the higher the extent of the aggregation cascade and thus a more significant change in visible colour is expected.

Intended Learning Outcomes

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

1. understand the operation of the testing and certification sector in Hong Kong and its contribution to food safety;
2. isolate melamine in milk and milk powder using suitable sample pretreatment

methods;

3. acquire the basic concepts of preparation and applications of nanomaterials;
4. understand the development of fast, low-cost, simple yet sensitive technique in analytical testing.

Experimental

Apparatus

- 1x 100-1000 μL auto pipette with pipette tubes or 1 mL graduated pipette
- 1x 10-100 μL auto pipette with pipette tubes or 0.2 mL graduated pipette
- 1x top pan balance
- 1x bench top centrifuge of at least 4000 rpm
- 4x PP centrifuge tube of 10 mL
- 5x PS cuvette
- 1x conical flask of 100 mL
- 1x stirrer hot plate
- 1x watch glass of 5 cm
- 1x volumetric flask of 25 mL
- 2x volumetric flask of 1 L
- 1x beaker of 100 mL
- 1x measuring cylinder of 100 mL
- 1x reagent bottle of 1 L
- 4x C18 SPE tube

Reagents and chemicals

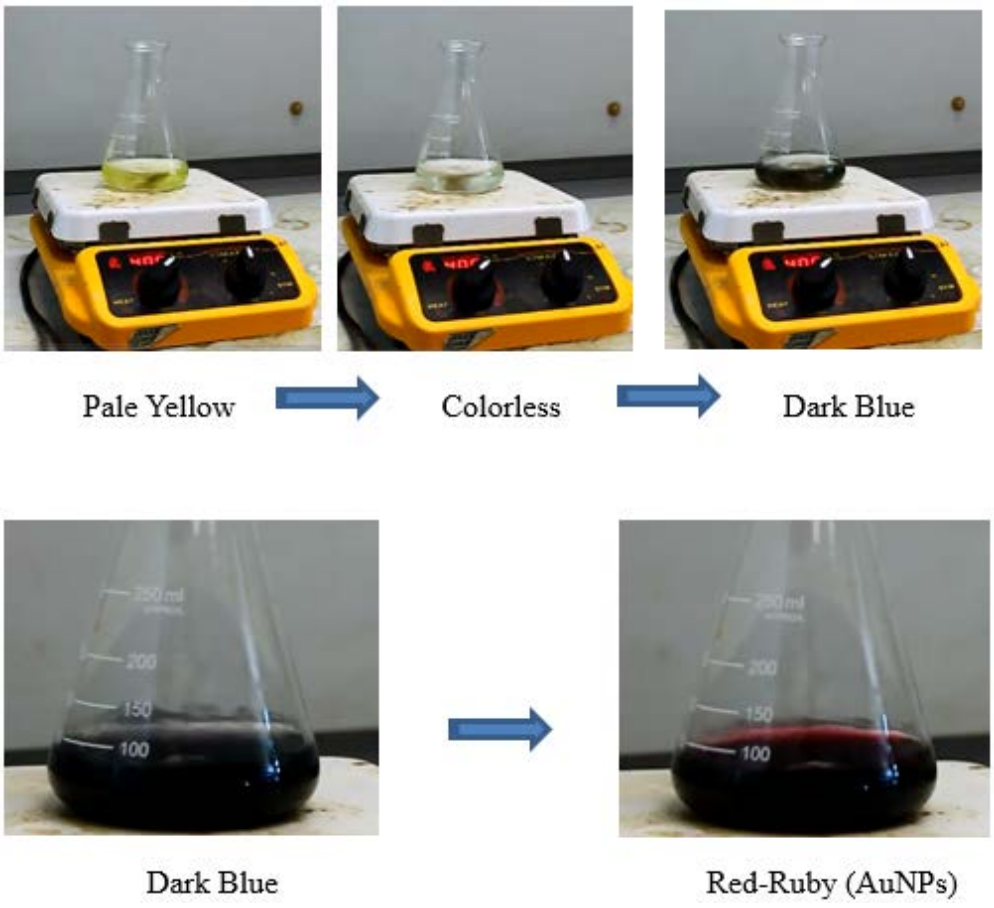
Description	Amount
0.1 M $HAuCl_4$	1 mL
38.8 mM sodium citrate	10 mL
10% TCA	10 mL
SPE elution solvent	20 mL
Chloroform	5 mL

Procedures

Part 1. Preparation of 13-nm Spherical Gold Nanoparticles (AuNPs)

Fig. 1. Set up for synthesizing 13 nm gold nanoparticles.

1. All glassware used are washed with aqua regia (3 parts HCl, 1 part HNO_3) and rinsed with filter Millipore water. The presence of dirt and grease affects the size of shape of the resulted nanoparticles.
2. The glassware to be used is oven-dried prior to use.
3. Prepare 50.0 mL of 1 mM $HAuCl_4$ by diluting of 500 μL of 0.10 M $HAuCl_4$ solution in water and boil in a 100 mL conical flask which is covered with a watch glass (see Fig. 1).
4. When the solution boils, rapidly add 5.0 mL of 38.8 mM sodium citrate to the stirring solution. Put some ice on the top of the watch glass to condense hot vapour.
5. Turn off the heater and continue stirring for 15 min., then cool down to room temperature.
6. This solution of gold nanoparticles will be used in the following experiments as the probe to melamine in samples. The following colour changes will happen within one minute.



Part 2. Fast screening the presence of melamine in dairy sample

A. Preparation of testing solution AuNPs

1. Pipette 5.00 mL of the freshly prepared AuNPs into a 25-mL volumetric flask.
2. Make up to the mark with D.I. water.

B. Sample pre-treatment and clean-up

a. For milk sample (liquid sample)

1. Pipette 500 µL sample into a 10-mL centrifuge tube which contains 7.50 mL D.I. water, 1.00 mL ~10% trichloroacetic acid (TCA), and 1.00 mL chloroform.

2. The function of TCA and chloroform is to precipitate the proteins and dissolve the fat existing in the milk sample, respectively.
3. Screw the cap and shake the tube vigorously to ensure the completeness of the extraction.
4. Repeat step 1.1 & 1.2 for a control sample (free of melamine).
5. Centrifuge the samples and set 4000 rpm for 10 mins. (see Fig. 2).
6. Label the centrifuge tubes according to the data sheet.

Fig. 2. Centrifuge tubes are placed diagonally so that mass is well-balanced.

b. For Milk powder (formula)

1. Mix 0.1 gram milk powder with 1.00 mL D.I. water. Pipette 500 μL sample into a 10-mL centrifuge tube which contains 7.50 mL D.I. water , 1.00 mL ~10% trichloroacetic acid (TCA), and 1.00 mL chloroform.
2. Screw the cap and shake the vial vigorously.
3. Repeat step 1.1 & 1.2 for a control sample (free of melamine).
4. Centrifuge the samples and set 4000 rpm for 10 mins.
5. Label the centrifuge tubes according to the data sheet.

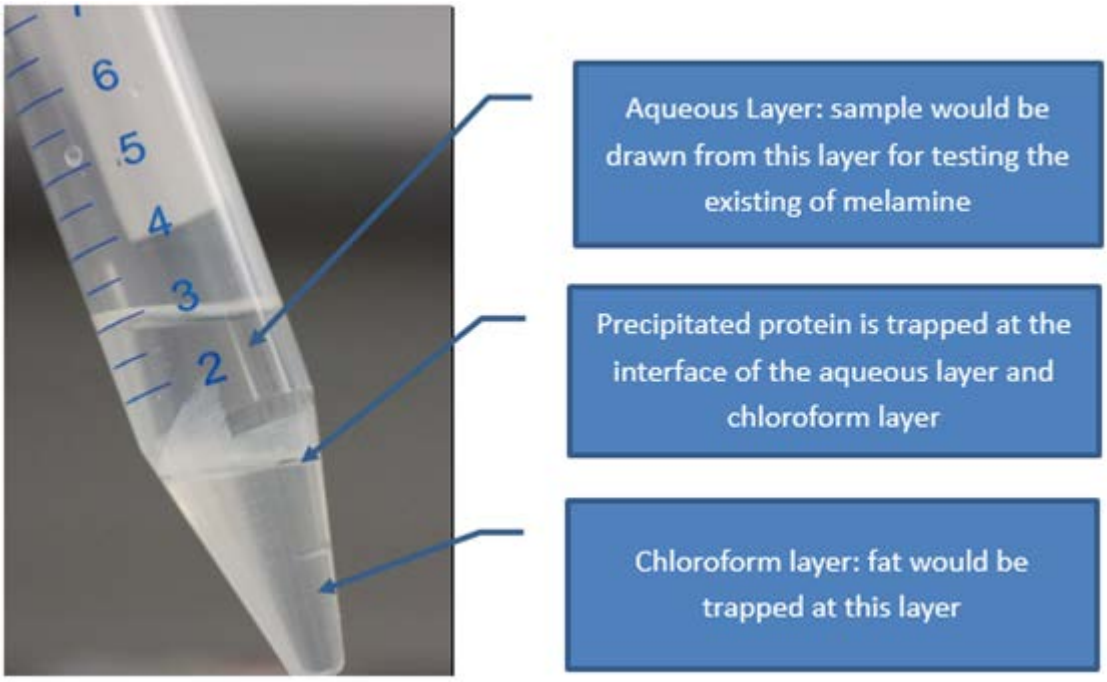
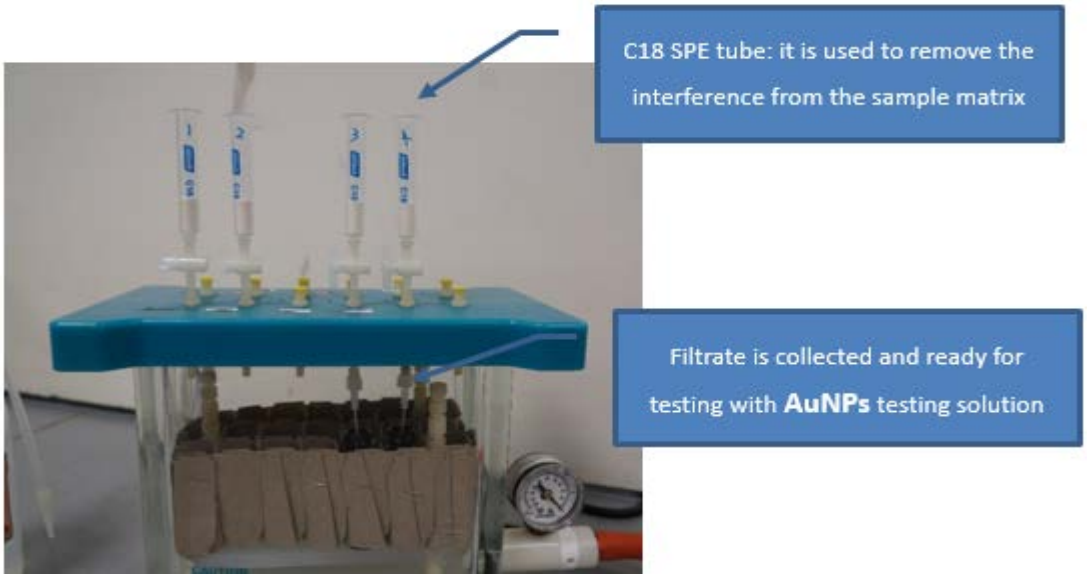


Fig. 3. After the centrifuge treatment, protein is precipitated out from the aqueous solution.

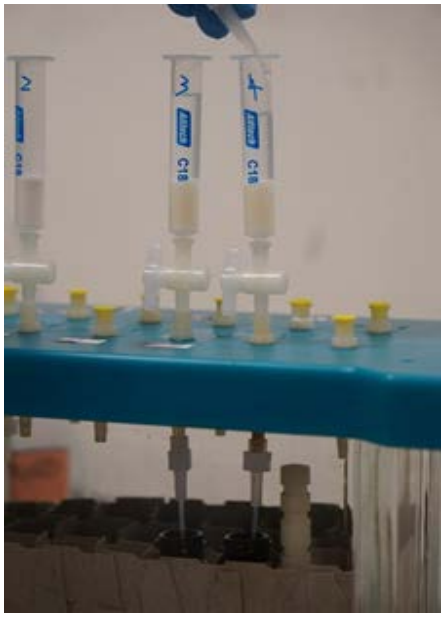
c. To clean up the supernatant using C18 SPE tube

1. Put a four C18 **S**olid **P**hase **E**xtraction (SPE) tubes on the vacuum manifold and label properly.

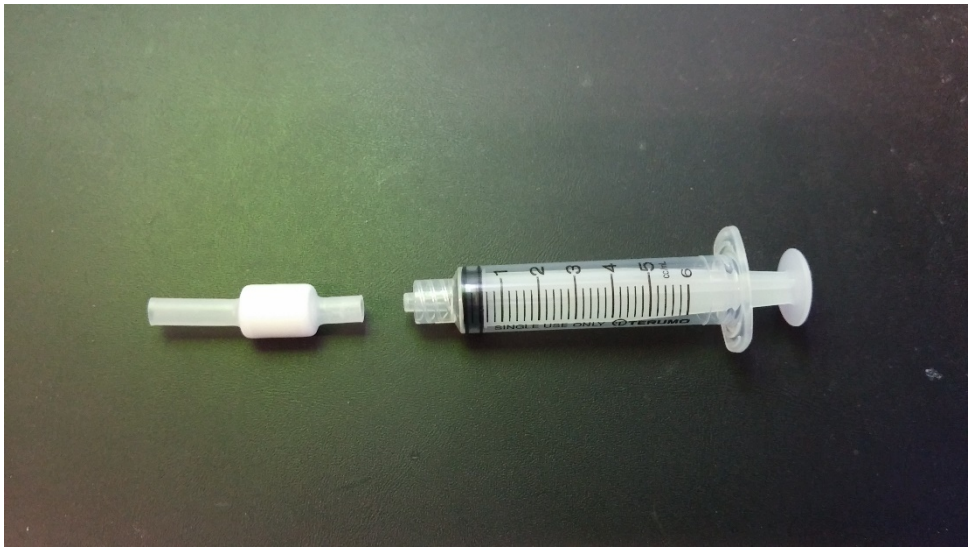


2. C18 SPE tube is wetted with 2 mL x 3 acetonitrile and then conditioned with 2 mL x 2 (1:1 acetonitrile/water).

3. Supernatant (aqueous portion) is transferred from the centrifuge tube to the C18 SPE tube.



4. A clean vial is put under the SPE as shown in the above photo.
5. Start the vacuum and open the tap.
6. Collect the filtrate at the clean vial.
7. Step #1 to #4 are repeated for sample # 1 to sample # 3.



In the absence of vacuum manifold, a disposal plastic syringe can be used to rinse the SPE tube (on the left) and push the supernatant solution through the SPE tube to be used in subsequent step shown below.

C. Screening test

1. Mix 100 μL filtrate with 2.00 mL AuNPs testing solution to observe the colour changes.
2. Compare the colour changes between samples. Record the colour changes and intensity in the data sheet.
3. Fill out the results in the data sheet.

Fig. 4. AuNPs testing solution (left); milk sample mixed with AuNPs without melamine (middle); milk sample mixed with AuNPs in the presence of 10 ppm melamine (right).

Safety precaution

- Observe the standard safety procedures for laboratory activity
- Put on the safety goggles, laboratory coats, and gloves
- Be careful when handling corrosive chemicals, such as concentrated acids
- Material Safety Data Sheet (MSDS) of chemicals are available online on the website of MSDSonline.com

Questions

1. State the colour changes from Gold (III) solution to AuNPs solution.

2. What is the function of sodium citrate in the formation of AuNPs?

3. What is the function of C18 SPE tube?

4. Do you think the use of AuNPs as a probe for melamine in milk samples would be a good method for melamine analysis?

Data Sheet

Sample #	Name of sample (if any)	Nature of sample	Vol. of sample added (mL)	Vol. of D.I. added (mL)	Vol. of 10% TCA (mL)	Vol. of $CHCl_3$ (mL)	Colour Changes/ Positive	Result positive or negative
1		**Liquid**	**0.5**	**7.50**	**1.00**	**1.00**		
2		**Liquid**	**0.5**	**7.50**	**1.00**	**1.00**		
3		**Solid**	**0.5***	**7.50**	**1.00**	**1.00**		
4		**Solid**	**0.5***	**7.50**	**1.00**	**1.00**		

*** weigh 0.1 g milk powder with 1.00 mL D.I. water**

Experiment 5 – Differentiation of Chinese Herb Danshen (丹參) from Other Similar Herbs Using Facile Test-Tube Scale Chemical Test Method

Introduction

Every day there are tons of Traditional Chinese Medicine (TCM) materials imported to Hong Kong. These herbal materials are distributed to thousands of Chinese medicine stores and sold in different ways. In order to identify these TCMs, a quick and facile scientific based chemical test has been developed. The approach is based on the identification of selected characteristic chemical constituents in TCM by test tube scale chemical reaction. Since a single herb may contain over tens or even hundreds of chemical components, the chemical functional groups of these compounds may react with certain specified reagents and produce various colours, precipitates, or crystals. By making use of the result of reactions, preliminary identification can be achieved. Facile chemical test involves mainly the observation of test-tube reaction. It refers to the observation of the expected chemical reactions between the extracted chemical components in TCM with the appropriate reagents in test tubes.

In our developed identification approach, the first step is to convert solid form TCM into powder form by blending. Powder form has a larger total surface area which will reduce the extraction time and increase the extraction efficiency. Then, a suitable solvent is used to extract the target chemicals like organic chemical components from the sample, e.g., diethyl ether. In general, the extraction efficiency can also be increased by increasing the temperature using hot water bath. If the chemicals are thermally unstable, ultrasonication can be used to improve the extraction yield. After extraction, centrifuge is used to separate the suspended solid from the solution. By spinning down the solid, the aqueous portion can be transferred to another clean and

empty new tube for further analysis. The changed colour and/or the presence of particles can be more easily observed.

In order to confirm the results, duplicate analysis for each sample is required. In addition, a positive control is also required in the experiment. It is used for comparing any colour or observational changes.

Radix Salviae Maltiorrhizae (Danshen), is an example to illustrate this technique for differentiation from closely-related herbs. Danshen is the dried root of *Salvia miltiorrhiza*, which is listed in the Pharmacopoeia of the People's Republic of China . It is commonly used for treatingmenstrual disorder and blood circulation problems, such as cardiovascular diseases. The chemical constituents of Danshen include both lipophilic and hydrophilic components. The major hydrophilic components are phenolic acids including danshensu (DSS), protocatechuic aldehyde (PA), rosmarinic acid (RA), and salvianolic acids, which are also the major pharmacologically active constituents[1].

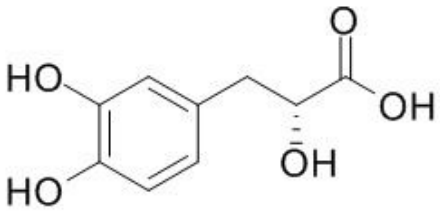


Fig. 1. Chemical Structure of Danshensu.

In general, the qualities and quantities of these compounds are analyzed with High Performance Liquid Chromatography (HPLC) with ultraviolet detector. However, it will take several hours to even days for the analysis to complete.

For the structure of Danshensu as shown in Fig. 1, it contains typical phenolic functional group. This characteristic group will react with iron (III) to form a green complex. Since these substances are water soluble, a hot water bath is used to extract it first and then tested by iron (III) chloride solution[2]. The formation of the dark green complex indicates the presence of phenolic functionality. This approach has been widely used in the TCM industry to test products claimed to be Danshen.

Intended Learning Outcomes

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

1. understand the operation of the testing and certification sector in Hong Kong and how it contributes to TCM development;
2. understand that modern techniques can be applied to TCM analysis;
3. isolate the chemical component in TCM using solvent extraction;
4. analyze the chosen analytes qualitatively using colour test.

Experimental

Apparatus

- 1x beaker of 50 mL
- 1x beaker of 1000 mL
- 1x beaker of 250 mL
- 1x volumetric flask of 100 mL
- 8x test tube
- 8x centrifuge tube of 15 mL
- 1x 100 – 1000 μL auto-pipette with pipette tubes or 1 mL graduated pipette
- 1x hot plate
- 1x centrifuge

- 1x spatula
- 1x balance

Reagents and chemicals

- 1,2-dihydroxybenzene
- Iron (III) chloride
- Deionized water

Lab preparation

1. Dissolve 0.33 g of iron (III) chloride hexahydrate ($FeCl_3 .6H_2O$) in 10 mL of D.I. water in a 50 mL beaker.
2. Dissolve 0.05 g of 1,2-dihydroxybenzene in 50 mL D.I. water and then dilute to the mark of a 100 mL volumetric flask as 500 ppm standard solution.
3. Purchase a Danshen sample (around 30 g) from a local store. Use a blender to breakdown the solid samples into powder form.

Sample pretreatment and Analytical methods

1. Compare the appearance of the samples.
2. Use a blender to breakdown the solid samples into powder form (Fig. 2).

Fig. 2. A blender is used to breakdown the solid sample into powder.

3. 0.1 g of powdered sample is mixed with 4 mL of D.I. water in centrifuge tubes.
4. Heat the mixture in a water bath for 15 min and then cool down.
5. Use centrifuge to spin down the solid sample.
6. Transfer 1 mL of the supernatant to a new test tube.
7. Add 0.1 mL of $FeCl_3$ indicator solution to each test tube.
8. Record the colour change of each test tube.

Safety precaution

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

Data Sheet

Morphological appearance

	Sample A	**Sample B**	**Sample C**
Shape			
Colour			
Colour of powder form			

Color of the extracted solution

Test tube	Content	Colour
1	D.I. water	
2	Sample A (trial 1)	
3	Sample A (trial 2)	
4	Sample B (trial 1)	
5	Sample B (trial 2)	
6	Sample C (trial 1)	
7	Sample C (trial 2)	
8	Chemical standard	

Colour of the extracted solution mixed with 0.1 mL $FeCl_3$ solution

Test tube	Content	Colour
1	D.I. water	
2	Sample A (trial 1)	
3	Sample A (trial 2)	
4	Sample B (trial 1)	
5	Sample B (trial 2)	
6	Sample C (trial 1)	
7	Sample C (trial 2)	
8	Chemical standard	

Questions

1. Why is a blender used to pretreat the sample?

2. Suggest another method to extract the water soluble chemicals from Danshen.

3. Why do we need a control test (D.I. water only)?

4. Why do we need to duplicate the experiment?

5. After mixing with $FeCl_3$ solution, explain the difference in observed colour change between samples and chemical standard.

6. Does Hong Kong has official standards of TCM (中藥材)? Can the standards serve the purpose of identifying whether a TCM is Danshen? (Hint : Find out about "Chinese Materia Medica" on the website of the Department of Health)

References

[1] Jin-zhong XU, Jie SHEN, Yi-yu CHENG, Hai-bin QU, J*ournal of Zhejiang University SCIENCE B*, ISSN 1673-1581 (Print); ISSN 1862-1783 (Online).

[2] www.cmd.gov.hk/hkcmms/vol1/Docs/radix_salviae_miltiorrhizae.pdf

Experiment 6 – Determination of Vitamin C in Commercial Sample of Fresh Fruit Juice by Iodometric Titration

Introduction

Vitamin C (L-ascorbic acid) is essential to our health. A high level of vitamin C is naturally found in citrus fruits and berries; while vegetables and edible animal internal organs such as liver and kidney contain lower levels of vitamin C. The human body is unable to synthesize vitamin C on its own and must depend on diet intake for an adequate supply. Nowadays, caplets, tablets, capsules, drink mix packets containing vitamin C as dietary supplement are available in the market. It is recommended from The National Academy of Sciences for a daily consumption of 60 mg vitamin C in order to meet the nutritional requirements of a healthy individual.

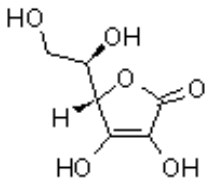
Vitamin C plays an important role in immune function. Insufficient vitamin C causes muscles weakness, swollen and bleeding of gums, loss of teeth and bleeding under the skin as well as tiredness and depression.

There are many methods that can be applied to determine vitamin C in fruits or vitamin supplement. Herein iodometric titration is applied to determine the amount of vitamin C in either fresh fruits or dietary supplement in tablet form. Since iodine is not a primary standard, it can be generated by mixing acidified iodate solution with iodide ions (1). The solubility of iodine is increased by complexation with iodide to form triiodide (2). Triiodide then oxidizes vitamin C to dehydroascorbic acid (3). The fast reaction reconverts iodine to iodide immediately when it is generated. When the limiting agent vitamin C is exhausted, the remaining iodine forms a dark blue complex with starch.

$$2\ IO_3^- + 10\ I^- + 12\ H^+ \longrightarrow 6\ I_2 + 6\ H_2O \quad (1)$$

$$I_{2\,(aq)} + I^- \rightleftharpoons I_3^- \quad (2)$$

$$C_6H_8O_6 + I_3^- \longrightarrow C_6H_6O_6 + 3\ I^- + 2\ H^+ \quad (3)$$



L-Ascorbic acid

Intended Learning Outcomes

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

1. understand the operation of the testing and certification sector in Hong Kong;
2. isolate vitamin C from fresh fruit samples using suitable sampling techniques;
3. analyze vitamin C quantitatively using iodometric titration method;
4. acquire the basic concepts of accuracy and precision of analytical methods.

Experimental

Apparatus

- 1x burette of 50 mL
- 1x burette clamp
- 1x stand
- 2x pipettes of 25 mL
- 1x measuring cylinder of 100 mL
- 2x beakers of 600 mL
- 1x beaker of 1 L
- 2x conical flasks of 250 mL
- 2x volumetric flasks of 250 mL

Reagents and chemicals

- Potassium iodide
- Potassium iodate
- Sulfuric acid
- L-ascorbic acid (Vitamin C)
- Starch

Fig. 1. Reagents required.

Lab preparation

- 5.00 g potassium iodide
- 0.300 g potassium iodate
- 30 mL 3 M sulfuric acid
- 0.250 g L-ascorbic acid
- 10 mL 1% starch solution
- 2 L deionized water

Sample pretreatment method

<u>With a fresh orange</u>

1. Slice the orange in half.
2. Grip the one of the orange halves tightly and squeeze it by hand, using a plain juicer to coax all the juice out (approximately 100 mL).

<u>With a Vitamin C tablet</u>

1. Dissolve one tablet of Redoxon into 100 mL of deionized water.
2. Dilute the solution to 1000 mL with deionized water.

Analytical method

Preparation of 0.01 M iodine solution

1. Weight approximately 5.00 g potassium iodide and 290 mg potassium iodate into a 600 mL beaker.
2. Add 200 mL deionized water to dissolve the mixture.
3. Add 30 mL of 3 M sulfuric acid.
4. Add 270 mL deionized water to the mixture.

Preparation of 1000 ppm vitamin C standard solution

1. Dissolve 0.250 g vitamin C in 100 mL deionized water.
2. Dilute to volume in a 250 mL volumetric flask.

Standardization of the iodine solution with the vitamin C standard solution

1. Pipette 25.00 mL of vitamin C solution into a conical flask of 250 mL and add several drops of 1 % starch solution.
2. Titrate the solution with iodine solution until the solution mixture changes to permanent blue colour.
3. Record the volume of iodine solution used and calculate the real concentration of the iodine solution (standardization).
4. Repeat the steps of 1 – 3 two times more in order to obtain the mean and standard deviation of the real concentration of iodine solution.

Fig. 2. Burette filled with iodine solution

Fig. 3. Addition of starch solution

Determination of vitamin C content in tablet by titration

1. Dissolve one vitamin C tablet into 1000 mL deionized water in a 1 L beaker.
2. Pipette 25 mL of the resulted vitamin C solution into a conical flask of 250 mL and several drops of 1 % starch solution.
3. Titrate the solution with the standardized iodine solution until the solution mixture changes to permanent blue colour.
4. Repeat the steps of 1 – 3 two times more.

Determination of vitamin C content in fresh fruits by titration

1. Measure 25 mL freshly squeezed juice by a measuring cylinder of 100 mL and transfer it to a conical flask of 250 mL.
2. Add several drops of 1 % starch solution and titrate the solution with iodine solution until the endpoint is reached.
3. Repeat the steps of 1 – 2 two times more.

Safety precaution

- Observe the standard safety procedures for laboratory activity
- Put on the safety goggles, laboratory coats, and gloves
- Be careful when handling corrosive chemicals, such as concentrated acids
- Be careful when cutting the fruit with a knife
- Material Safety Data Sheet (MSDS) of chemicals are available online on the website of MSDSonline.com

Data Sheet

Weight of potassium iodide = _____ g

Weight of potassium iodate = _____ g

Weight of pure vitamin C = _____ g

Total volume of the freshly squeezed juice = _____ mL

Standardization of the iodine solution with the vitamin C standard solution

	Trial 1	Trial 2	Trial 3
Initial burette reading (mL)			
Final burette reading (mL)			
Volume of iodine solution used (mL)			

The number of moles of ascorbic acid used in each trial = _____ mol

The average volume of iodine solution used = _____ mL

∴The real concentration of the iodine solution = _____ M

Titration of vitamin C tablet solution

	Trial 1	Trial 2	Trial 3
Initial burette reading (mL)			
Final burette reading (mL)			
Volume of iodine solution used (mL)			

The average volume of iodine solution used = _____ mL

The average number of moles of iodine used = _____ mol

The average number of moles of vitamin C = _____ mol

The mass of vitamin C content determined (mg) = _____ mg

∴ The vitamin C content of tablet (ppm) = _____ ppm per tablet

Titration of fresh fruit juice

	Trial 1	Trial 2	Trial 3
Initial burette reading (mL)			
Final burette reading (mL)			
Volume of iodine solution used (mL)			

The average volume of iodine solution used = _____ mL

The average number of moles of iodine used = _____ mol

The average number of moles of vitamin C = _____ mol

The mass of vitamin C content (mg) = _____ mg

∴ The vitamin C content of fresh fruit juice (mg) = _____ mg

Questions

1. How much vitamin C is there in a vitamin C tablet (mg)? Does the value agree with the label?

2. Is the selected fruit a good source of vitamin C? Explain.

3. Suggest another method that can be used to determine vitamin C.

4. Is vitamin C required to be identified on the nutrition labels for foods sold in Hong Kong? Do you think Hong Kong's commercial testing laboratories can test for all the items on the nutrition label?

References

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[4] GL-CO-33 method accessed from http://www.itc.gov.hk/en/quality/hkas/doc/scopes/001.pdf