“Teaching Kit on Chemical Testing for Senior Secondary Curriculum”

Teachers’ Guide

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

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.[1]

[1] 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.

Knowing the Testing and Certification Industry in Hong Kong

Background

The Hong Kong Council for Testing and Certification (HKCTC) was established in 2009 to advise the Government on the overall development strategy of the testing and certification sector. One of the core initiatives of the Council is to support the professional development of the testing and certification sector in Hong Kong.

Role and Profile of Hong Kong's Testing and Certification Sector

The testing and certification sector plays an important role in our daily life. The Government Laboratory provides a wide range of analytical, investigatory, and advisory services and support to enable the Government to meet its responsibilities in different areas such as law and order, public health and safety, and environmental protection. Private laboratories are playing an increasing role in the analysis of food, water, and environmental samples. Medical laboratories provide support to the medical sector in the diagnosis of illnesses. Construction 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 Pearl River Delta Region. 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. Through providing assurance to overseas buyers on the quality and safety of products, the sector is important in the economic development of Hong Kong and the Pearl River Delta Region.

Accreditation in Hong Kong

Accreditation is the third-party attestation related to a conformity assessment body conveying formal demonstration of its competence to carry out specific conformity assessment tasks. Laboratories, inspection bodies and certification bodies are common types of conformity assessment bodies.

Accreditation is open and voluntary in Hong Kong. It is currently provided by Hong Kong Accreditation Service (HKAS) under Innovation and Technology Commission in Hong Kong. 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).

Accredited laboratories, inspection bodies and certification bodies need to undergo rigorous on-site assessments before they are recognised to be competent in performing the conformity assessment activities listed in their respective scopes of accreditation. Users of conformity assessment services may identify and select the services provided by accredited bodies to support their business. 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).

Manpower Development in Testing and Certification Sector

Hong Kong's testing and certification sector is built upon a workforce of professionals with high integrity and technical expertise.

For testing laboratories and inspection bodies, staff can be non-degree holders but more and more have attained university level education. Relevant disciplines in universities are mainly science, applied science, engineering, fashion and textiles, etc. Certification bodies generally require persons with university level education and working experience in relevant trades. They may come from various academic disciplines. The sector is looking for people with good communication and language skills. Work knowledge is mainly built up through on-the-job training.

The tertiary education sector in Hong Kong has been offering a number of 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 HKCTC's website (www.hkctc.gov.hk/en/career/edu/course_list.html).

In addition, the Hong Kong Association for Testing, Inspection and Certification (HKTIC), a non-profit making trade association, offers certification to practitioners in different aspects including method validation, quality control, and instrument calibration.

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 industry to Hong Kong's economy and society. It may enhance student's interest in chemistry knowledge by bringing science closer to them.

Teacher's Guide

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

Introduction

On 1 January 2014, the Environmental Protection Department (EPD) of Hong Kong SAR Government introduced the new "Air Quality Health Index" (AQHI) to inform the public on the possible risks to health from exposure to different levels of air pollution in the outdoor environment. The calculation of the AQHI is based on the 3-hour average concentrations of four air pollutants: ozone, nitrogen dioxide, sulphur dioxide and particulate matter. Hourly analytical data of these pollutants collected from different air monitoring stations have been used to calculate the AQHI and the results are announced for public information.

Nitrogen dioxide (NO_2) is one of a group of highly reactive gases known as "oxides of nitrogen", or "nitrogen oxides (NO_x)". Other nitrogen oxides include nitric oxide, nitrous acid and nitric acid. The US Environmental Protection Agent's National Ambient Air Quality Standard uses NO_2 as the indicator for the larger group of nitrogen oxides whereas EPD establishes one of the air quality objectives based on NO_2 for pollution control management. NO_2 forms quickly from emissions from electricity generation, road transport, navigation, civil aviation and other fuel combustion sources. NO_x react with ammonia, moisture and other compounds to form small particles. These small particles penetrate deeply into sensitive tissues of the lungs and can cause or worsen respiratory diseases, such as emphysema and bronchitis, and aggravate existing heart disease, leading to increased hospital admissions and premature death. Furthermore, under sunlight and in the presence of volatile organic compounds (VOCs) such as hydrocarbons, nitric oxide has proved to be a culprit for promoting the

formation of the highly toxic photochemical smog.

$$\text{VOCs} + \text{NO} + \text{O}_2 + \text{sunlight} \rightarrow\rightarrow \text{mixture of } \text{O}_3, \text{HNO}_3, \text{and organics (smog)}$$

On the other hand, ground-level ozone is produced by the photochemical dissociation nitrogen dioxide initiated by sunlight.

$$\text{NO}_2 + \text{UV light} \rightarrow\rightarrow \text{NO} + \text{O} \quad ; \quad \text{O} + \text{O}_2 \rightarrow\rightarrow \text{O}_3 \text{ (ozone)}$$

When we breathe in high concentration ozone, ozone can cause serious health effects. People with lung disease children, older adults and people who are active in the outdoor environment may be particularly vulnerable to the effects of ozone. Due to the adverse health effects of the pollutant, government environmental protection departments have set limits for its ambient concentration as the air quality objective or standard. Quality objective for NO_2 in hourly average and annual average from Hong Kong, the USA and the EU [European Union] are given in Table 1.

Table 1. Air quality objective or standard for NO_2 (in $\mu g/m^3$ air: part per billion, ppb).

	Hong Kong	**USA**	**European Union**
1 hour average	200	100	200
Annual	40	53	40

NO_2 in ambient air can be continuously determined as the azo dye by forming the diazonium salt from sulfanilic acid and then coupling it with *N*-(1-naphthyl)-ethyldiamine dihydrochloride (*vide infra*, in the experimental part). The method is selective and sensitive in the order of parts per hundred million of air[1]. A facile

chemiluminescence reaction of luminol as shown in Fig. 1 with NO_2 is exploited for the precise determination of NO_2. The principle of measurement is based upon the reaction of luminol with NO_2 which results in the emission of light. The limit of detection (LOD) for NO_2 is approximately 50 parts per trillion (i.e., 0.05 ppb). Interferences from other gaseous air molecules such as NO, O_3, SO_2 and CO_2 to nitrogen dioxide determination are negligible[2].

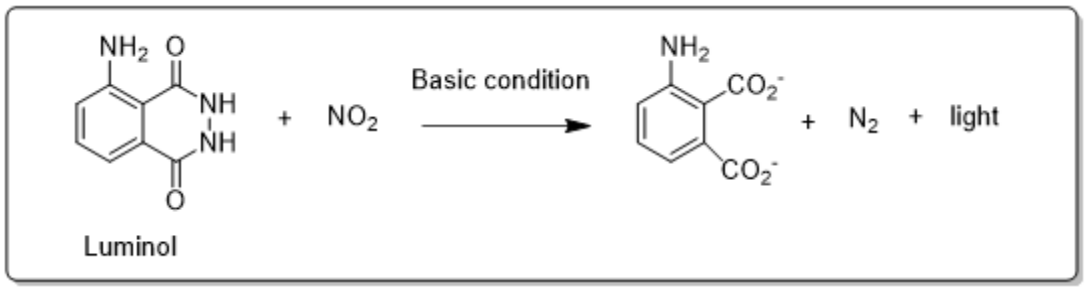


Fig. 1. Chemiluminescence method for the determination of NO_2.

Recently, commercial NO_2 gas analyzers are available for continuous monitoring of the ambient NO_2 level (Fig. 2). Air monitoring stations are equipped with such real time NO_2 analyzer for measuring the hourly average concentration of ambient NO_2. The air quality information is accessible from the website of EPD on hourly basis.

Fig. 2. Continuous NO_2 analyzer – a commercial model.

For illustration, the hourly ambient NO_2 concentration data on 1st January 2019 from

six regional air monitoring stations are tabulated in Fig. 3. Apparently, the ambient NO_2 content found in various stations fluctuated and all data comply well with the air quality objective (i.e., <200 ppb). In addition, the data obtained from the Mongkok Road Side Air Monitoring Station are consistently higher than those from other General Stations, especially during the daytime.

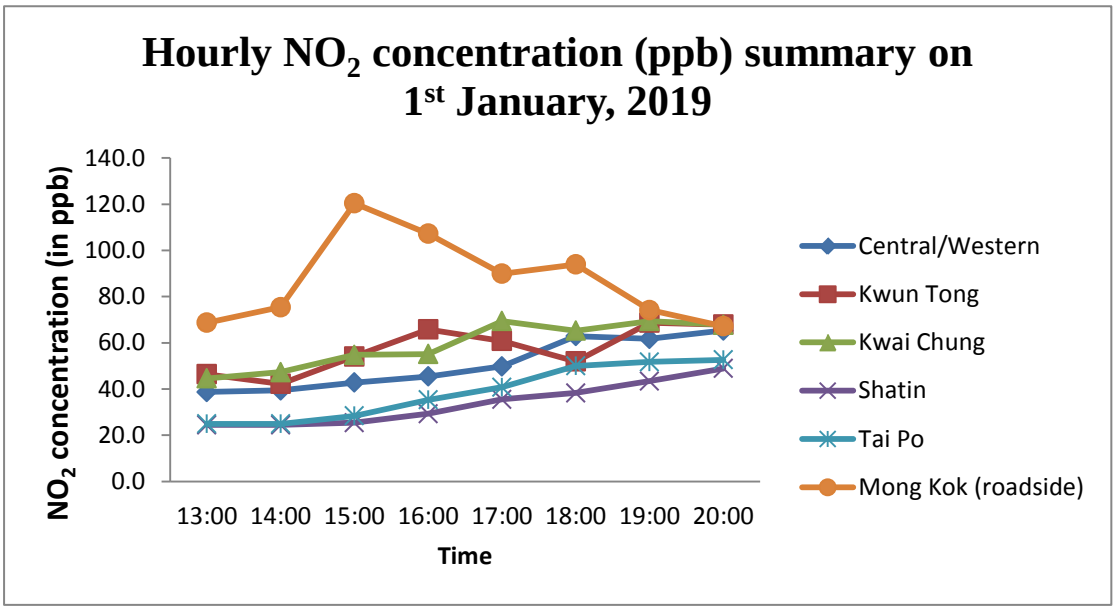


Fig. 3. Hourly NO_2 concentration (in ppb) summary on 1st January 2019 from selected air monitoring stations (from EPD website).

Perhaps the simplest means of actively collecting an air sample is by a constant flow rate sampling pump coupled with adsorbent device. Prior to the sampling, the constant flow-rate pump must be first calibrated with an air flow calibrator. For determining ambient NO_2, air is drawn through the sampling pump to a train of impinges containing colourless aqueous solution of *N*-(1-naphthyl)-ethyldiamine dihydrochloride and sulfanilamide. In contrast, gaseous pollutants such as NO_2 can diffuse into a passive sampler incorporated sorbent materials. Commercial passive sampling devices are available in market. In the former device, the intensity of the

colour change is determined by a photometer or is read on a scale or compared to a chart in order to determine concentration levels. In the latter device, the adsorbed materials can be desorbed and determined by colourimetric and other instrumental analytical methods. A handy passive sampling device, with Chinese Patent[3], which consists of a glass vial equipped with a Teflon membrane and fixed with a screw cap onto the vial was developed. Partially filled the vial with the absorbing solution and sealed with the gas diffusive membrane hold tightly by a screw cap, the device can serve as a passive sampler for gaseous pollutants including NO_2. Passive sampling device is low in cost and can be installed simultaneously at multi-sampling sites suitable for a comprehensive study. However, to provide quantitative results, an experimentally determined “sampling rate” or “uptake rate” must exist for each pollutant of concern with the sorbent sampling media. If both active and passive sampling devices are used simultaneously to determine the concentration level of an air pollutant at the same location for the same sampling duration, the active sampling device can be used to establish the uptake rate of a passive sampling device (see Fig. 4).

Fig. 4. Calibrating of the diffusion rate of the passive sampler by impinges with a constant flow pump.

The significance of analyzing atmospheric samples can't be overestimated especially in environmental analysis. However, in the analytical chemistry elective section of the current Senior Secondary Chemistry Curriculum, only the qualitative analysis of a few common gaseous molecules has been covered and discussed. Thus the principle and the content of this laboratory activity could confer teachers and students with important analytical knowledge and techniques on how to sample and quantify airborne materials. The technique acquired can be exploited easily to the determination of other pollutants such as formaldehyde, sulfur dioxide and nitric oxide.

Outdoor airborne NO_2 content has been continuously monitored by EPD. Indoor air quality (IAQ) in both household and office environment is equally important to health. Poor IAQ can lead to discomfort, low working efficiency and ill health. NO_2, generated in indoor from flue gas of burning stove and tobacco smoking, has been identified as one of the indoor pollutants. For promoting a healthy indoor environment, EPD has launched the IAQ Certification Scheme (www.iaq.gov.hk). Through the Hong Kong Laboratory Accreditation Scheme (HOKLAS)[4], a number of local testing laboratories have been accredited by HKAS for measuring NO_2 as one of the IAQ parameters. Most accredited laboratories are testing indoor air samples collected by the passive sampling method. Airborne 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, those instruments are not available in secondary schools. In this laboratory activity, on the basis of a literature procedure[1], a colourimetric method for the determination of NO_2 in both outdoor and indoor environment is used instead[5].

Recently, a very simple protocol for teaching colourimetry using smartphone was described[6]. 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. Since most of local secondary schools are only equipped with 1 or 2 colourimeter(s) in their chemistry laboratories, such a mobile app which is available for every student with smartphone can provide a hands-on experience for each of the students, greatly facilitating their learning of analytical chemistry. The application of "ChemEye" in the detection of NO_2 in air will be demonstrated in this experiment. Moreover, the in-app calculation and conversion have simplified the steps of manual calculation and graph sketching, which users can easily obtain the calibration curve and information of sample content. The learning experience of the students has been enriched by this simple, handy and convenient method. In comparison with the 4-LED photometer, the performance of "ChemEye" is similar in terms of repeatability and accuracy of less than 5% difference. Furthermore, in terms of sensitivity to detect the target analyte, the performance is even better than that of 4-LED photometer for 3 times.

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 colourimetry;
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 [121-57-3]
- 0.025 g *N*-(1-naphthyl)-ethylenediamine dichlorohydrate [1465-25-4]
- 5 mL 1-propanol [71-23-8]
- 0.75 g sodium nitrite [7632-00-0]
- 2 L Deionized water

Sample pretreatment method

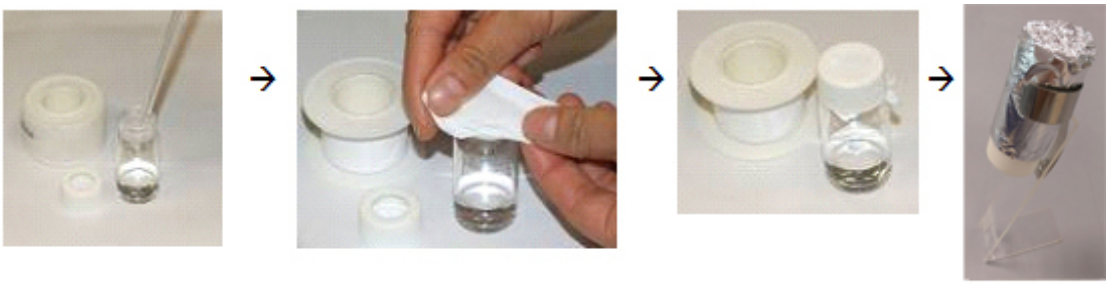
1. Set-up of gas samplers
    i. Pipet 5 mL of absorbent reagent into the sampling vial (i.e., sampler).

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. After 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 minutes 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

Results and Discussion

Preparation of the absorption solution and calibration with standards

To avoid the absorption of airborne NO_2 during the preparation of absorbent reagent, the whole process must proceed rapidly. The colourless absorption solution after its preparation should be kept closed in the volumetric flask with an air-tight stopper. The colourimetric method has been used to determine the NO_2 concentration in air. Air containing NO_2 can be drawn by a pump or going through the passive sampler (demonstrated in this activity) to a colourless absorbing solution containing two organic reagents [i.e., sulphanilic acid (**A**) and *N*-(1-naphthyl)-ethylenediamine (**B**)]. NO_2 is highly soluble in water to give nitrous acid and nitric acid. Nitrous acid (HNO_2) can react with **A** and **B** to give a red azo dye product. Higher the concentration of nitrous acid in the absorbing solution, deeper the red colour will be –

Reaction 1: $2\ NO_2$ (from air) + $H_2O \rightarrow\rightarrow HNO_2$ (aqueous) + HNO_3 (aqueous)

Reaction 2: HNO_2 (aqueous) + reagent A + reagent B $\rightarrow\rightarrow$ a red colour product

(colourless absorbing solution) (red colour solution)

To establish the relationship between the intensity of the red colour and the concentration of nitrite (i.e., $-NO_2^-$), standard nitrite solutions covering the concentration range from 0.02 – 0.12 ppm are prepared for performing the calibration experiment. By using a colourimeter, the absorbance of different standard solutions is measured. 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.

Air sampling

Under normal conditions, 1.5 – 2 h sampling time may be required to allow sufficient NO_2 to diffuse into the sampling vial, triggering detectable colour change for an accurate quantification of the pollutants. To ensure the precision of the measurement, at least three sets of air samplers are to be placed at the same sampling location inside the school campus for collecting air samples during the same period of time (i.e., 90 or 120 min). For a comparison study, another 3 sets of sampling vials can be placed at a site close to the traffic (outside campus). To acquire reliable results, it is advisable to instruct the students to perform the air sampling step well ahead of the laboratory period such as during the recess prior to the laboratory class.

For purpose of illustrative, two sampling sites in the Hong Kong Baptist University (HKBU) Campus were chosen:

1. Site 1: along Waterloo Road side, 6/F podium in HoSinHang Campus
2. Site 2: near 12/F lift lobby in Science Tower, HoSinHang Campus

Three trial samplers and one control were placed in close proximity at each of the sampling sites.

Construction of calibration curve by 4-LED photometer

In this laboratory activity, a 4-LED colourimeter will be used. The light intensity measured (i.e., absorbance) by the colourimeter can be calculated as shown in Table 2. When the green LED light source is used, the absorbance (A) of the red colour solution can be deduced from the logarithm of the detected potential between the blank and the standard colour solution.

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

Absorbance = $\log\frac{E_o}{E}$ using **green** LED measurement, E_o = __3.61__ V

Table 2. Detected potential and absorbance of the standard solutions determined by the 4-LED colourimeter.

Descriptions	**E (V)**	**Absorbance**
Blank	E_o = 3.61	0
0.02 ppm NO_2 standard	3.50	0.013
0.04 ppm NO_2 standard	3.43	0.022
0.08 ppm NO_2 standard	3.24	0.047
0.12 ppm NO_2 standard	3.06	0.072

On the basis of these data, a linear calibration curve correlating the intensity of the solution and the concentration of nitrite (or airborne NO_2) can be constructed.

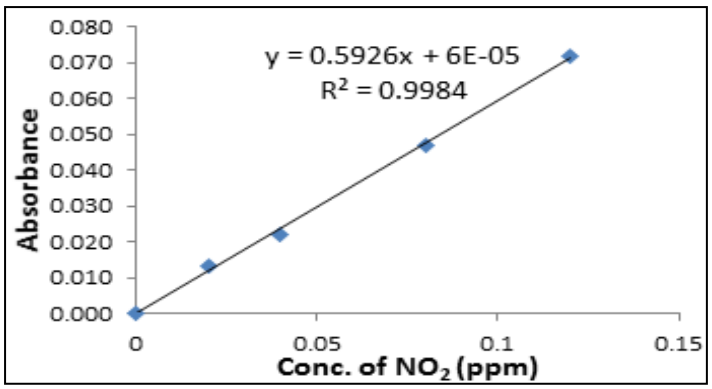


Fig. 5. Calibration curve of the standard nitrite (NO_2) solution.

The linearity of the calibration curve of Fig. 5 is very good as the R^2 value is of 0.998. The calibration curve is suitable to be used for the real-life sample determination.

Airborne NO_2 determination by 4-LED photometer

Again, the 4-LED colourimeter was used for absorbance measurements.

Absorbance = $\log \frac{E_o}{E}$ using **green** LED measurement, E_o = ___3.61___V

Sampling time: ___90___ min

The air diffusion rate was determined by the active impinge method as described in the previous section. The sampling rate of the passive sampler has been found to be: 2.3×10^{-5} m^3/min, which will be used in the study of all partner schools in this project.

Table 3. Detected potential of the sampling solutions at the two sites in the Ho Sin Hang Campus of Hong Kong Baptist University[a]

Sample	E (V)			
	1st trial	2nd trial	3rd trial	mean
Roadside (sample)	3.49	3.48	3.47	3.48
Roadside (control)				3.56
6/F campus podium (sample)	3.51	3.52	3.51	3.51
6/F campus podium (control)				3.58
12/F campus lift lobby (sample)	3.53	3.53	3.53	3.53
12/F campus lift lobby (control)				3.57

[a] The sampling was conducted at 10:00 – 11:30 on 20 June 2015, a sunny day.

Data treatment by 4-LED photometer

1. Calculate the absorbance (A) for the standards and sample solution.

   The absorbance of 0.02 ppm NO_2 standard: log (3.61/3.50) = 0.013

Descriptions	E (V)	Absorbance
Blank	E_o = 3.61	0
0.02 ppm NO_2 standard	3.50	0.013
0.04 ppm NO_2 standard	3.43	0.022
0.08 ppm NO_2 standard	3.24	0.047
0.12 ppm NO_2 standard	3.06	0.072
Roadside (sample)	3.48	0.016
Roadside (control)	3.56	0.006
6/F campus podium (sample)	3.51	0.0122
6/F campus podium (control)	3.58	0.0036
12/F campus lift lobby (sample)	3.53	0.0097
12/F campus lift lobby (control)	3.57	0.0048

2. Based on the calibration curve constructed in Fig. 5, the concentration of the sampling and controlled solutions can be obtained and the results are shown in Table 4 below.

Table 4. Concentration of NO_2 found in the samplers at different sites.

	E (V)	**Absorbance**	**Conc. of NO_2 (mg/L)**
Roadside (sample)	3.48	0.016	0.027
Roadside (control)	3.56	0.006	0.010
6/F campus podium (sample)	3.51	0.0122	0.020
6/F campus podium (control)	3.58	0.0036	0.007
12/F campus lift lobby (sample)	3.53	0.0097	0.017
12/F campus lift lobby (control)	3.57	0.0048	0.008

3. Calculate the weight of NO_2 collected in the sampler and the control:

   (Use data collected from roadside samplers as the calculation example)

   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

   = 0.027mg/L $\times$ 10^3 μg/mg $\times$ 5 mL $\times$ 10^{-3} L/mL

   = 0.135 μg

   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

   = 0.010 mg/L $\times$ 10^3 μg/mg $\times$ 5 mL $\times$ 10^{-3} L/mL

   = 0.05 μg

   Net weight of NO_2 collected (μg):

   Weight of NO_2 in sampler (μg) - Weight of NO_2 in control (μg)

   = 0.135 μg – 0.05 μg = 0.085 μg

4. Calculate the volume of air sample (m^3):

   Volume of air sample (m^3) = Diffusion rate (m^3/min) × sampling time (min)

   = 2.3x 10^{-5} m^3/min $\times$ 90 (min)

   = 0.00207 m^3

5. Calculate the concentration of NO_2 in atmosphere with unit of $\mu g/m^3$:

NO_2 ($\mu g/m^3$) = weight of NO_2 (μg) / volume of air sampled (m^3)

= 0.085 μg / 0.00207 m^3

= 41 $\mu g/m^3$

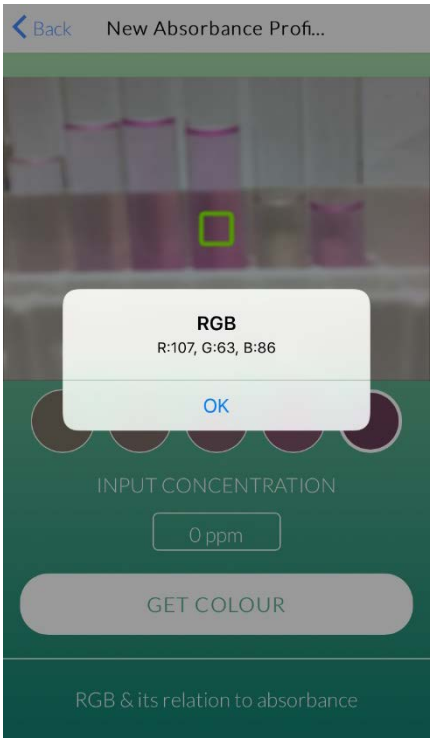
By using similar calculation steps, the concentration of NO_2 of the other two sites can be calculated and they are shown in Table 5.

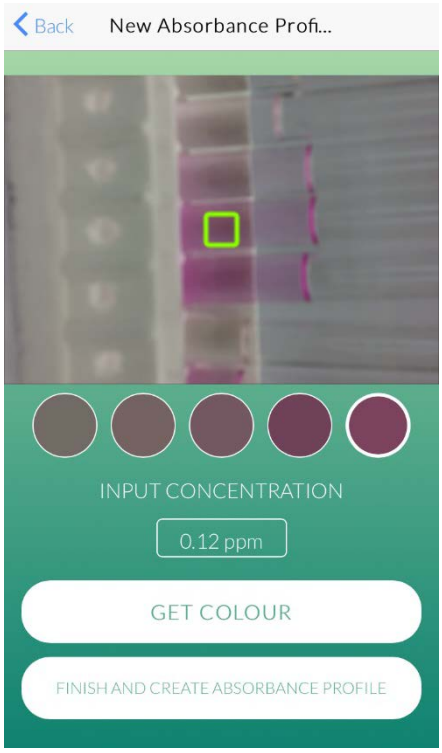
Table 5. Concentration of airborne NO_2 found at three sampling sites in HKBU detected by 4-LED photometer.

	Weight of NO_2 in sampler (μg)	**Weight of NO_2 in control (μg)**	**Net weight of NO_2 collected (μg)**	**Volume of air sample (m^3)**	**Conc. of NO_2 in atmosphere ($\mu g/m^3$)**
Road side	0.135	0.05	0.085	0.00207	41.0
6/F campus podium	0.1	0.035	0.065	0.00207	31.4
12/F campus lift lobby	0.085	0.040	0.045	0.00207	21.7

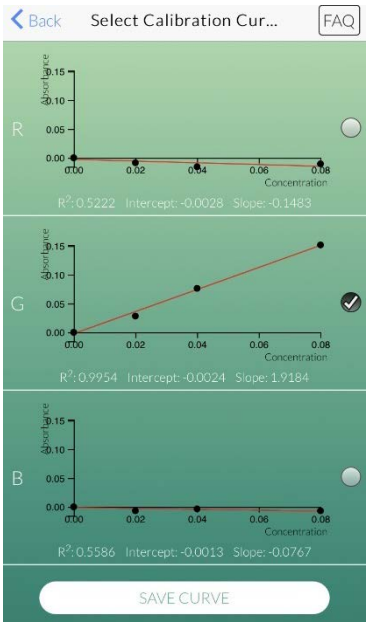
Measurement by ChemEye

In this part of the laboratory activity, ChemEye installed in the mobile device will be used, replacing the use of 4-LED photometer. Camera of the mobile device is used as detector for target analyte solution. The R, G and B value in the selected area of the image will be recorded. The pixel value captured in the image will be analyzed and converted to absorbance of red, green and blue color.



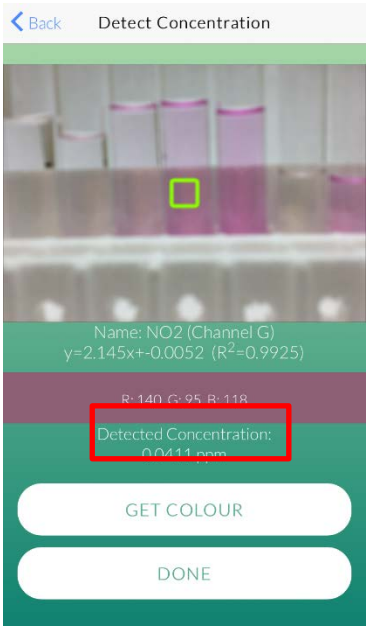


Capture the R, G and B value of the five standard solutions by the camera and plot the calibration curve.



After recording five images of blank and the respective standard solutions, the mobile app will automatically convert the R, G and B values obtained from the images to absorbance. The calculated absorbance will be used to plot three respective calibration curves with slope, y-intercept and linear regression coefficient (R^2) shown in the calibration page. Users can choose the curve by their own to save in the profile. In this experiment, the curve of G-value has the steepest slope and the greatest linear regression coefficient (R^2) among three curves, then it will be used in the further analysis of sample.

Data treatment by ChemEye



After creating the calibration curve, sample can be immediately detected by the ChemEye. The concentration of the sample is calculated by the app with saved equation and shown in the detection page directly. Conversion of NO_2 content in atmosphere can refer to the "Data treatment by 4-LED photometer". The calculation steps are the same after getting the concentration of NO_2 in the collected solutions by "ChemEye".

Conclusion

Colourimetry, being one of the most essential instrumental analytical methods, is covered in the HKDSE Chemistry Elective topic (i.e., analytical chemistry). With suitably designed reagents, the method using the colourimetry principle can be developed to analyze a wide variety of chemical species, including gaseous molecules. The formation of coloured product by the absorbent reagents used in this activity is rapid and highly selective to NO_2. Among common airborne molecules, only NO_2 can react with the reagents. Through this activity, the students can acquire the technique of undertaking passive air sampling without too much difficulty. In addition, the analytical method described is extremely sensitive and the detection limit is in the sub ppm (i.e. part per million) level. 90 – 120 min sampling time is sufficient for collecting detectable airborne NO_2. With some tactful arrangement for installing the gas samplers beforehand, the teacher can complete this laboratory activity within 2 laboratory class periods. The data obtained in the campus environment can be compared with the on-line air pollution information available on EPD's website. If teachers are interested to guide the students to analyze airborne NO_2 as one of the IAQ

parameters, they should consult the document "A Guide on Indoor Air Quality Certification Scheme for Offices and Public Places" accessible in the website of IAQ (https://www.iaq.gov.hk/en/publications-and-references/guidance-notes.aspx). For measuring indoor airborne NO_2, 8 hour of sampling time is normally required. The real life relevant nature of the activity will be appealing and treasured by students. The activity confers students with a quantitative knowledge on one of the major air pollutants in our environment.

Questions and Answers

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

   After the sampler has stood near road side for two hours, the absorbent reagent changes from colourless to pink.

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

   The pink colour complex is absorbed in wavelength around 550 nm which is the green light region.

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

   Active sampler usually requires shorter sampling time and airborne gaseous materials are pulled into the absorbent reagent by a pumping system.

   Passive sampler requires relatively long sampling time and airborne gaseous materials are penetrating into the absorbent reagents through the membrane by diffusion.

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

   The absorbent reagent is easily decomposed by sunlight.

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?

After collecting NO_2 gas in aqueous solution, the generated nitrite ion can be determined by flow injection method or ion chromatographic method. These two methods, requiring the use of special instruments, are sensitive and selective.

After Class Group Work

1. Visit the website of EPD to locate the relevant data obtained from different General and/or Road Side Monitoring Station, and then make a comparison with the data obtained in the laboratory.
2. Go to the HOKLAS webpage to find out the list of commercial testing laboratories that can provide testing of NO_2 as an IAQ parameter.

References

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[3] 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.
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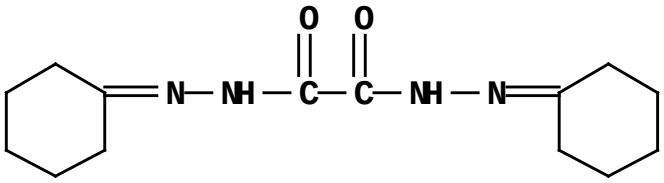
Experiment 2 – Analysis of Copper in Wastewater

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 "colourimetric" 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, colourimetric 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 colourimeter 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.

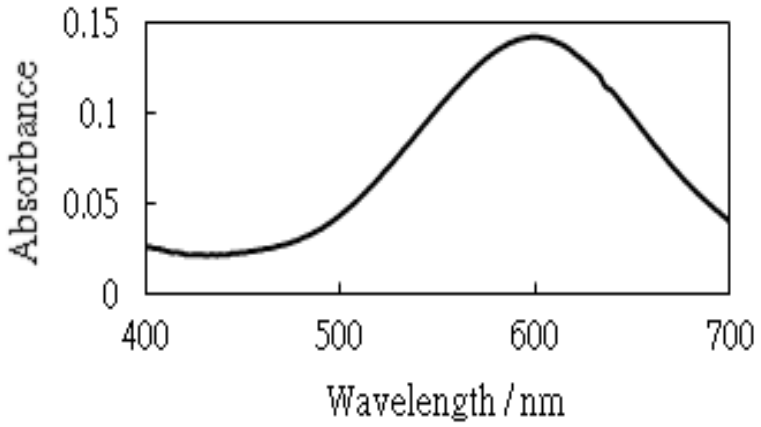


Fig. 1. Visible spectrum of Cu (II)-Cuprizone.

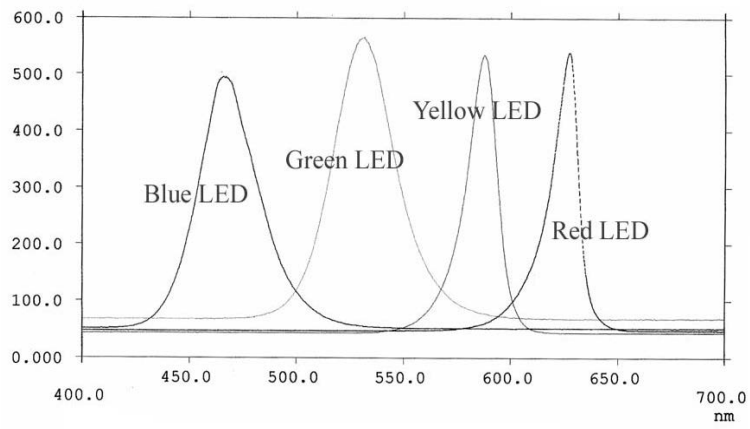


Fig. 2. Emission spectrum of four coloured LED.

The topic "Basic Principles and Applications of Colourimetry" is included in the analytical chemistry elective section of the current Senior Secondary Chemistry Curriculum. This laboratory activity provides teachers and students with important analytical knowledge and techniques on how to quantify metal species, such as copper, by employing a colourimetric reagent to convert the analyte to a coloured compound that can be determined by colourimetric technique. The technique acquired can be applied to the determination of other metal species such as lead and nickel. In addition, spectrophotometer is recommended to be in the standard list of teaching equipment by the Education Bureau (EDB).

In this laboratory activity, a colourimetric method is employed for the determination of copper content in industrial effluents and wastewater. The instruments utilized, usually colourimeters or spectrophotometers, are available in secondary schools. On the other hand, metal contents are frequently determined by other spectrochemical techniques, such as atomic absorption spectrophotometry (AAS)[4], inductively coupled plasma – atomic emission spectrometry (ICP-AES)[5], or inductively coupled plasma – mass spectrometry (ICP-MS), in local accredited testing laboratories.

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 colourimetry;
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 plastic cuvette (for 4-LED photometer detection)
- 6x test tubes (for ChemEye detection)
- 1x test tube rack (for ChemEye detection)
- 1x hot plate
- 1x 4-LED photometer and/or mobile device installed with "ChemEye"

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

Reagent and chemicals

- Oxalic acid bis(cyclohexylidene hydrazide) [370-81-0]
- Copper (II) sulfate [7758-98-7]
- Ethanol [64-17-5]
- Citric Acid [77-92-9]
- 25 % Ammonia Solution [7664-41-7]

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

   <u>4-LED photometer</u>

   I. Procedure

   i. Turn on the power of the colourimeter, turn on the <u>Yellow LED</u> 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.

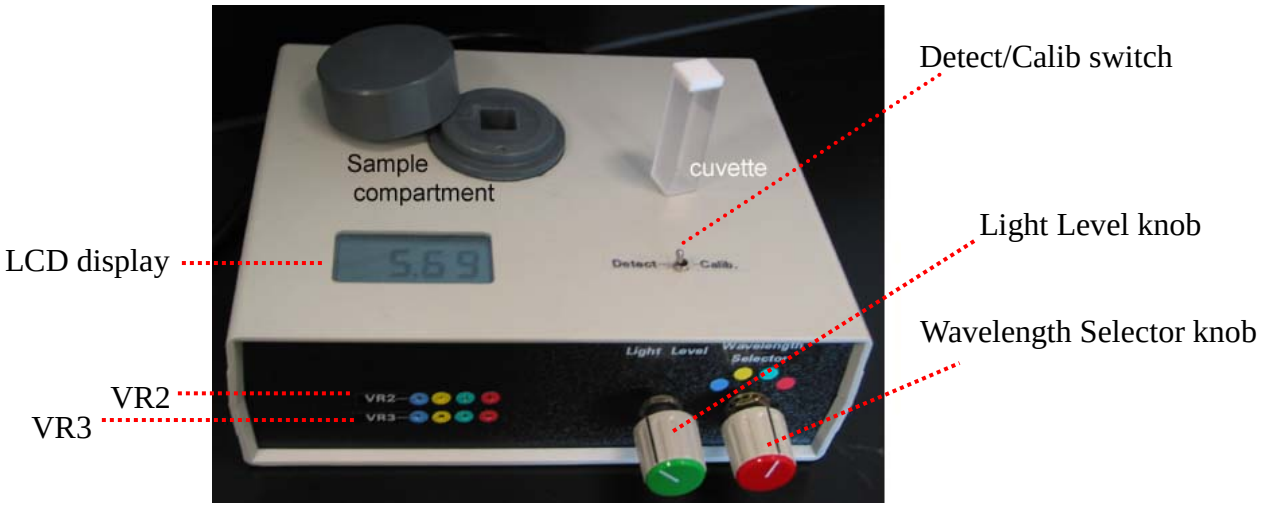
   v. Remove the cover of cell holder.

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

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

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

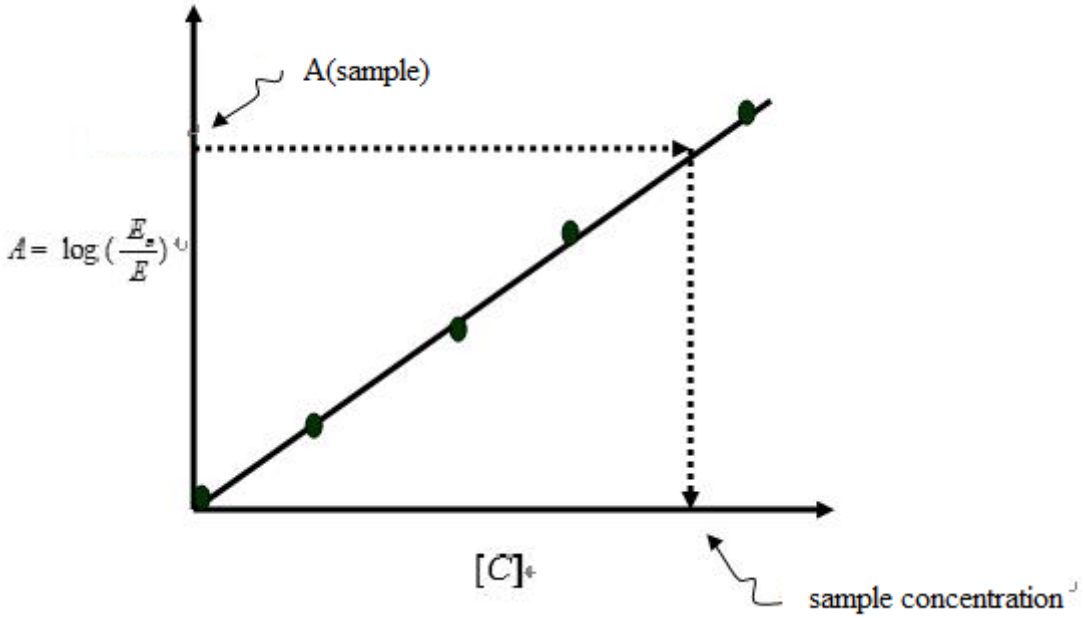
where E is the potential measured with the sample placed in the sample holder,

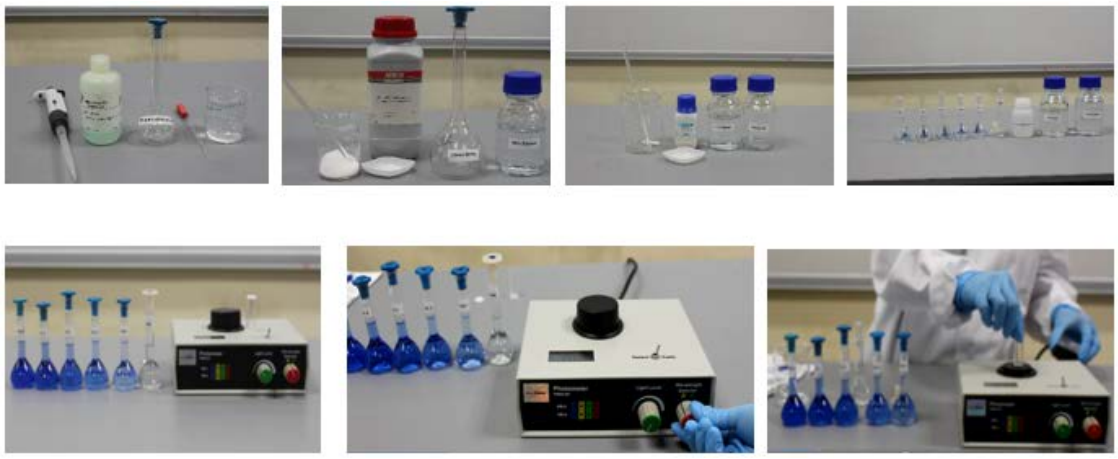
A is Absorbance

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)

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

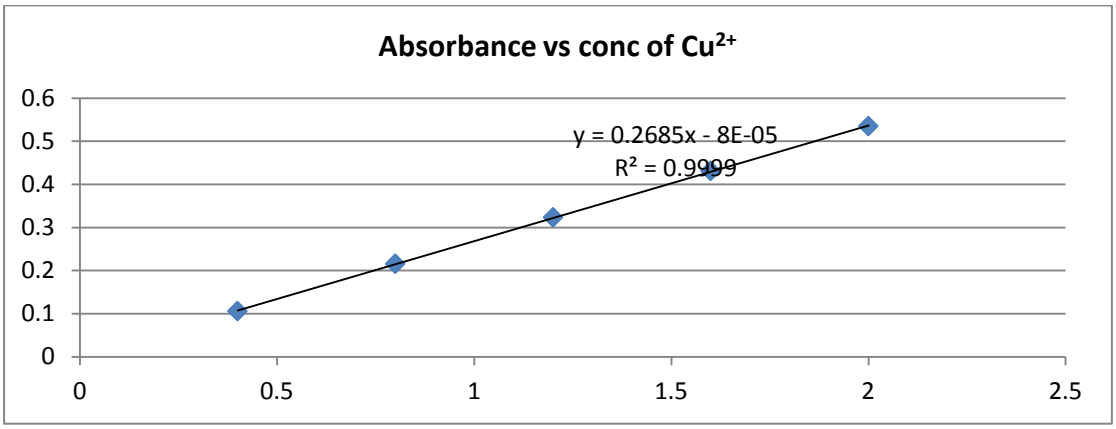




Data Sheet for 4-LED photometer (yellow LED):

Calibration Voltage =			
	(E)	$\log\left(\frac{E_\circ}{E}\right)$	[Cu] /ppm
Blank	E_o = 5.45	0.000	0.000
Standard #1	4.27	0.1060	0.4
Standard #2	3.32	0.2152	0.8
Standard #3	2.59	0.3231	1.2
Standard #4	2.02	0.4310	1.6
Standard #5	1.59	0.5350	2.0
Sample	2.55	0.3298	1.23
Linear coefficient (R^2) = 0.9999			

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 for iOS device or from PlayStore for Android device.

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 points 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.

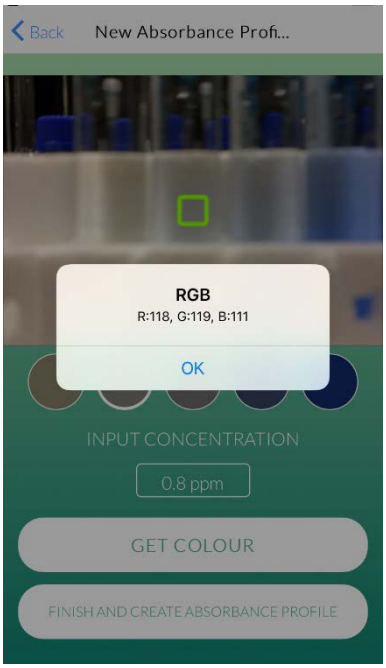
vii. Repeat the step above for the sequence from the lowest concentration to the highest (i.e.:0 ppm, 0.80 ppm, 1.20 ppm, 1.60 ppm and 2.00 ppm).

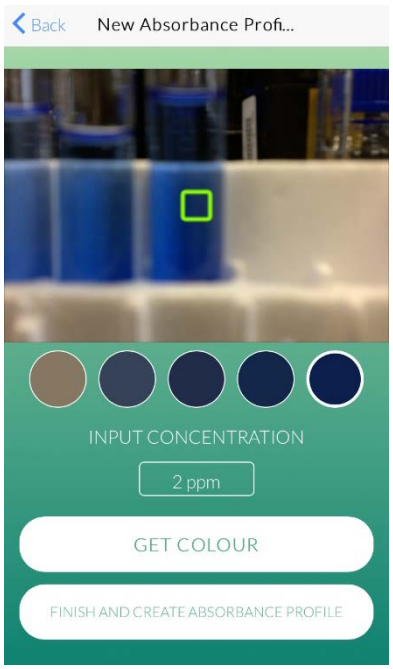
viii. Input all the information required for constructing the calibration curve. Press "Finish and create absorbance profile".

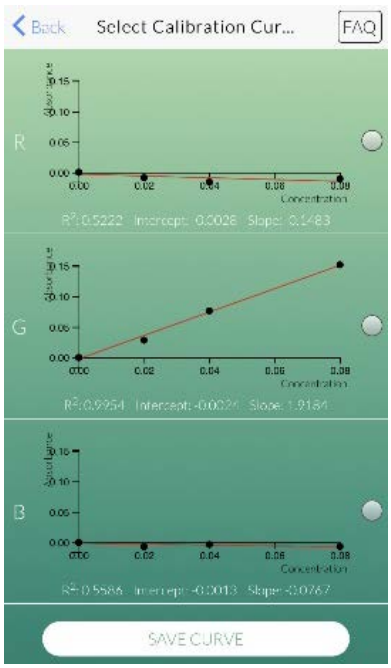
ix. Three calibration curves will be generated from converting the RGB value captured to absorbance. From the FAQ section, there are some guidelines for selecting which curve best fit to the detection of analyte.

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.

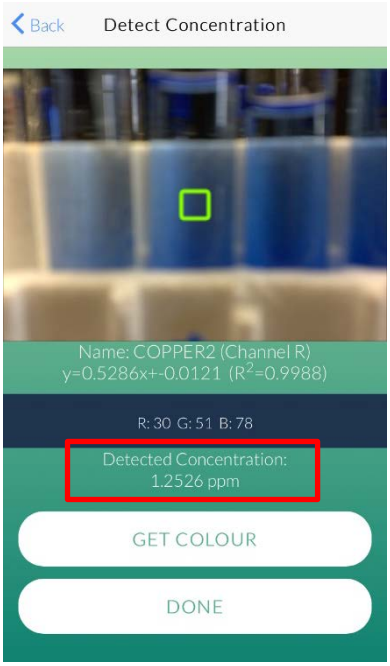






II. Data and data treatment

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.



Data Sheet for ChemEye:

1. Which curve have you chosen for the calibration? R, G or B value?
   G value
2. Please state the reason(s) of choosing the respective graph as the calibration curve?
   Among the three calibration curves, the G-value curve has the steepest slope which means having the highest intensity of absorbance in green color by the cuprizone color complex.
   On the other hand, the linear regression is the highest which means the relationship of absorbance against concentration has highest linearity.
3. What is the slope and linear coefficient (R^2) of the calibration curve selected?
   (Copy from the app)
4. What is the concentration of copper in the wastewater?
   (Will be shown after sample detection)

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

Observation

A blue colour is observed when cuprizone is added to samples and standard solutions containing copper (II).

Conclusion

Determination of toxic metal ion content in water samples including drinking water and industrial wastewater is important in daily life. A wide variety of analytical reagents have been developed for the colourimetric determination of toxic metal such as Pb (II), Cd (II) and Cu (II). This experiment provides an example of the analytical procedure to analyse metal content in water samples. By choosing a suitable colorimetric agent for Pb^{2+}, the lead ion concentration in water can be determined with a similar procedure.

Questions and Answers

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

   zinc, tin, silver, etc.

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?

   Cuprizone reacts with Cu^{2+} to form the colour complex which absorbs visible light. Cu^{2+} does not absorb visible light to a good extent. Without the formation of colour complex in the presence of cuprizone, Cu^{2+} cannot be determined.

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

   The blue colour complex has maximum absorption at around 600 nm and does not absorb as much radiation when a green LED is used. The measurement sensitivity (or the slope of the calibration curve) will be lower when a green LED is employed.

References

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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.

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.

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. Natural food stores generally carry food that have not been treated with sulphur dioxide. However, this type of food is often darker in colour and may have a different texture from those treated with sulphur dioxide. Furthermore, this type of food will not stay suitable for consumption as long as those treated with sulphur dioxide and needs to be consumed soon.

Since sulphur dioxide in food may have an impact on human health, this experiment is designed to students of Secondary 5 or above, who have the basic knowledge of chemistry in senior secondary school level, for quantitative determination of the sulphur dioxide content in dried food. The experiment can easily be carried out during a typical two-hour laboratory period in a senior secondary school laboratory and does not require any advanced instrumentation. In the current Senior Secondary Chemistry Curriculum, the volumetric analysis of acid-base titration has been fully covered. It is also required to equip the students with the knowledge of application of appropriate tests for detecting the presence of sulphur dioxide and the contribution of analytical chemistry to society in the analysis of food and drugs in the elective section of

Analytical Chemistry. The principle and the content of this experiment could confer teachers and 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 test.

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 a bubbler to the receiver containing 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).

Quantitative determination of the sulphur dioxide content in food is beneficial to the public health. The determination is, therefore, 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. In this experiment, students will be able to learn one of the methods of AOAC Official Method 990.28.

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.

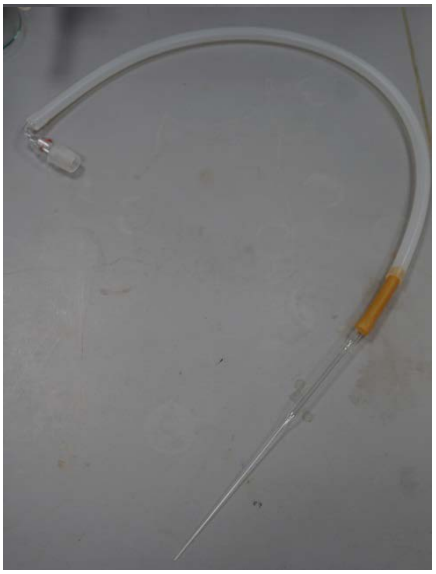


Fig. 2. Gas collecting tube.

Reagents and chemicals

- Ethanol [64-17-5]
- Hydrochloric acid (HCl) [7647-01-0]
- Hydrogen peroxide (H_2O_2) [7722-84-1]
- Methyl red [493-52-7]
- Phenolphthalein [77-09-8]
- Potassium hydrogen phthalate (KHP) [877-24-7]

- Sodium hydroxide (NaOH) [1310-73-2]

Lab preparation

- 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. Dried food sample of Chinese Yam.

Fig. 4. 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. 5)
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. 5. Sample in the two necked round bottom flask.

6. Assemble the setup as shown in Fig. 6.
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. 6. 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 vapors
- Material Safety Data Sheet (MSDS) of chemicals are available online on the website of MSDSonline.com

Results and Discussion

Observation

1. During the heating of 60 minutes, gas is bubbled into the 3% hydrogen peroxide solution. The colour of the solution changes from yellow to red. (Fig. 7)
2. At the end point of the titration of standardization of sodium hydroxide solution, the colour of the solution changes from colourless to pink.

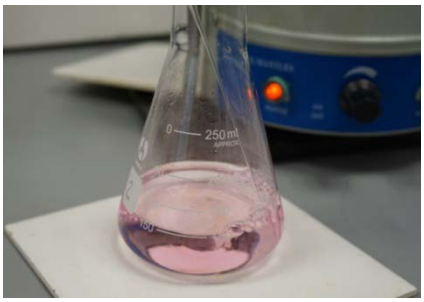


Fig. 7. Bubbling gas into the 3% hydrogen peroxide solution.

3. At the end point of the titration of determination of sulphur dioxide content, the colour of the solution changes from red to yellow.

Data and data treatment

Standardization of sodium hydroxide solution

	1st trial	**2nd trial**	**3rd trial**
Mass of KHP (g)	0.0500	0.0515	0.0518
Final volume (mL)	29.7	38.8	39.3
Initial volume (mL)	0.20	7.00	8.9
Volume of NaOH used (mL)	29.5	31.8	30.4

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

∴ Mole ratio of NaOH: KHP = 1:1

	1st trial	**2nd trial**	**3rd trial**
Number of mole of KHP (mol)	2.45×10^{-4}	2.52×10^{-4}	2.54×10^{-4}
Number of mole of NaOH (mol)	2.45×10^{-4}	2.52×10^{-4}	2.54×10^{-4}
Concentration of NaOH (M)	0.00831	0.00792	0.00836

- [number of mole of KHP (mol)] = [mass of KHP (g)] / [molar mass of KHP (g/mol)]
- molar mass of KHP = 204.22 g/mol
- [concentration of NaOH (M)] = [number of mole of NaOH (mol)] / [volume (dm^3)]

∴ The real concentration of the sodium hydroxide solution = $\underline{0.00820 \pm 0.000241\ M}$

Sodium hydroxide solution must be standardized against an acid, such as potassium hydrogen phthalate, of known molarity prior to the use as titrant. Sodium hydroxide salt often contains significant amount of insoluble sodium carbonate, which leads to overestimation of the concentration of sodium hydroxide in a solution. Furthermore, sodium hydroxide solution can react with dissolved or atmospheric carbon dioxide to form sodium carbonate, which leads to loss of sodium hydroxide in the solution. The concentration of sodium hydroxide in a solution would be lower than the expected value. If standardization is not performed, there will be errors in the measurement.

Determination of sulphur dioxide content by titration

	Chinese Yam
Mass of dried food sample (g)	12.6540
Final volume (mL)	10.0
Initial volume (mL)	5.3
Volume of NaOH used (mL)	4.7

H_2O_2 (aq) + SO_2 (g) → H_2SO_4 (aq)

2NaOH (aq) + H_2SO_4 (aq) → Na_2SO_4 (aq) + $2H_2O$ (l)

∴ Mole ratio of NaOH: SO_2 = 2:1

	Chinese Yam
Number of mole of NaOH (mol)	3.85×10^{-5}
Number of mole of SO_2 (mol)	1.93×10^{-5}
Mass of SO_2 (g)	1.24×10^{-3}
SO_2 content (mg/kg)	98.0

- [number of mole of NaOH (mol)] = [concentration of NaOH (M)] x [volume (dm^3)]
- As shown above, the real concentration of the sodium hydroxide solution = 0.00820 M
- [mass of SO_2 (g)] = [number of mole of SO_2 (mol)] x [molar mass of SO_2 (g/mol)]
- molar mass of SO_2 = 64.1 g/mol
- [SO_2 content (mg/kg)] = [mass of SO_2 (g) x 1000] / [mass of dried food sample (g) / 1000]

∴ The sulphur dioxide content of Chinese Yam = <u>98.0 mg/kg</u>

The sulphur dioxide content of Chinese Yam is much higher than the value listed in the regulation of 10 mg/kg. The functional class of sulphur dioxide and the corresponding name shall be, therefore, specified in the list of ingredients if the Chinese Yam is sold in the market in Hong Kong.

Possible measurement using advanced instrumentation

According to AOAC Official Method 990.29, the sulphur dioxide content of food can be determined by flow injection analysis (FIA) using reaction with malachite green. This method is applicable for all kinds of food, except dried food. Sulphur dioxide is first extracted from the food sample by sodium tetrachloromercurate (TCM) solution. The extract is then injected into the FIA system, where sulphur dioxide will react with malachite green and cause discolouration of malachite green (Fig. 8). The degree of discolouration is proportional to the amount of sulphur dioxide in the food sample.

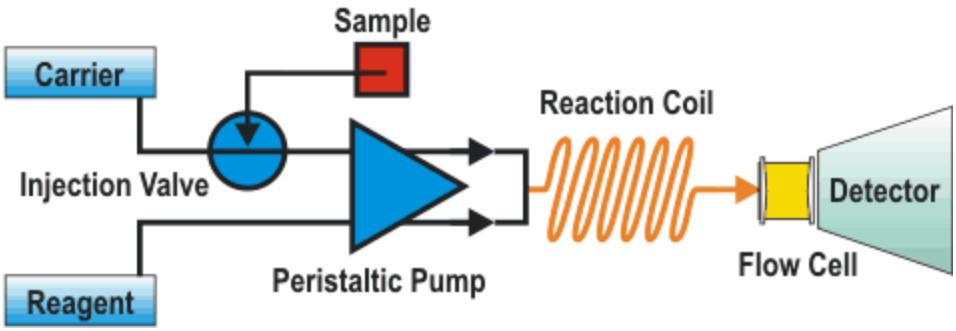


Fig. 8. Systematic diagram of a flow injection analysis system.

Conclusion

Acid-base titration, which is one of the most important technique in wet analysis, has been fully covered in the Senior Secondary Chemistry Curriculum. The technique can be applied for quantitative determination of the sulphur dioxide content in food sample that has been appropriately treated first. Although the heating time in sample pretreatment requires 60 minutes, the experiment can be completed in a two-hour laboratory period with tactful arrangements in setting up the apparatus beforehand. Through the experiment, students can acquire the use of reflux setup to isolate gaseous species from solid sample and the concept of conversion of the analyte into a suitable form for measurement, i.e., sulphur dioxide is converted into sulfuric acid. The data obtained in the experiment can be checked against regulatory controls, which helps to develop the student awareness of sulphur dioxide in food and also interest in food analysis. Since the experiment is designed based on a standard method that is widely applied in the testing and certification sector, students can develop a basic understanding on the corresponding operation in Hong Kong.

Questions and Answers

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.

   Infrared (IR) spectroscopy with gas sample cell. SO_2 has a strong absorption band at wavenumber of 1300 – 1400 cm^{-1}.

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

   Salts of nitrite and nitrate can act as preservative in food, but they can react with amines in food to form nitrosamines which are carcinogens.

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

   Soap the food in water. SO_2 is water soluble and become H_2SO_4.

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

   Nitrates and nitrites; benzoic acid, sorbic acid.

References

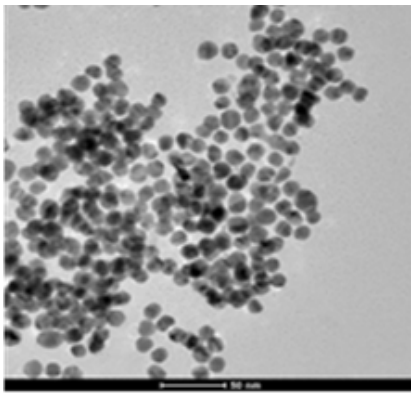
[1] AOAC International: *Official Methods of Analysis of AOAC* (2005), 18th Edition., ed. Horwitz W., Gaithersburg: MD, Method 990.28.

[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 the 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, interest of students will be brought to nanomaterial chemistry and food analysis, which are covered in the Senior Secondary Chemistry Curriculum of Material and Analytical Chemistry, respectively. 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 clear 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.

After the incident of milk products being contaminated by melamine was exposed, local HKAS accredited testing laboratories have started offering melamine testing. Their service can help the milk industry to meet relevant regulation in Hong Kong as

well as those imposed by other economies in the world, including the United States of America and the European Union.

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

- 25 mL 0.1 M Chloroauric acid trihydrate [27988-77-8]
- 1 L 38.8 mM Sodium citrate [6132-04-3]
- 1 L 10% Trichloroacetic acid (TCA) [76-03-9]
- 24 mL Chloroform ($CHCl_3$) [67-66-3]
- 750 mL Concentrated Hydrochloric acid (HCl) [7647-01-0]
- 250 mL Concentrated Nitric acid (HNO_3) [7697-37-2]
- 250 mL Acetonitrile (ACN) [75-05-8]

Lab preparation

Stock solutions are prepared according to the table below:

Stock#	Description	Chemical used	Amount	D.I. used	Remark
1*	0.1 M $HAuCl_4$	Chloroauric acid trihydrate	1.0 g	25.0 mL	It should be stored at 4°C after used
2	38.8 mM sodium citrate	sodium citrate dihydrate	11.45 g	1.00 L	Stored at room temperature
3	10% TCA	Trichloroacetic acid	105.5 g	1 L	
4	Aqua regia**	750 mL HCl + 250 mL HNO_3 in a 1L reagent bottle			
5	SPE elution solvent	250 mL ACN + 250 mL D.I. Water in a 500 mL reagent bottle			

* Stock#1 to 5 could be prepared once and served for the whole class of students.

** After cleaning with Aqua regia, the solution could be reserved and reused about five times.

For a single set of experiment, the above mentioned stock solutions are distributed to students according to the table below:

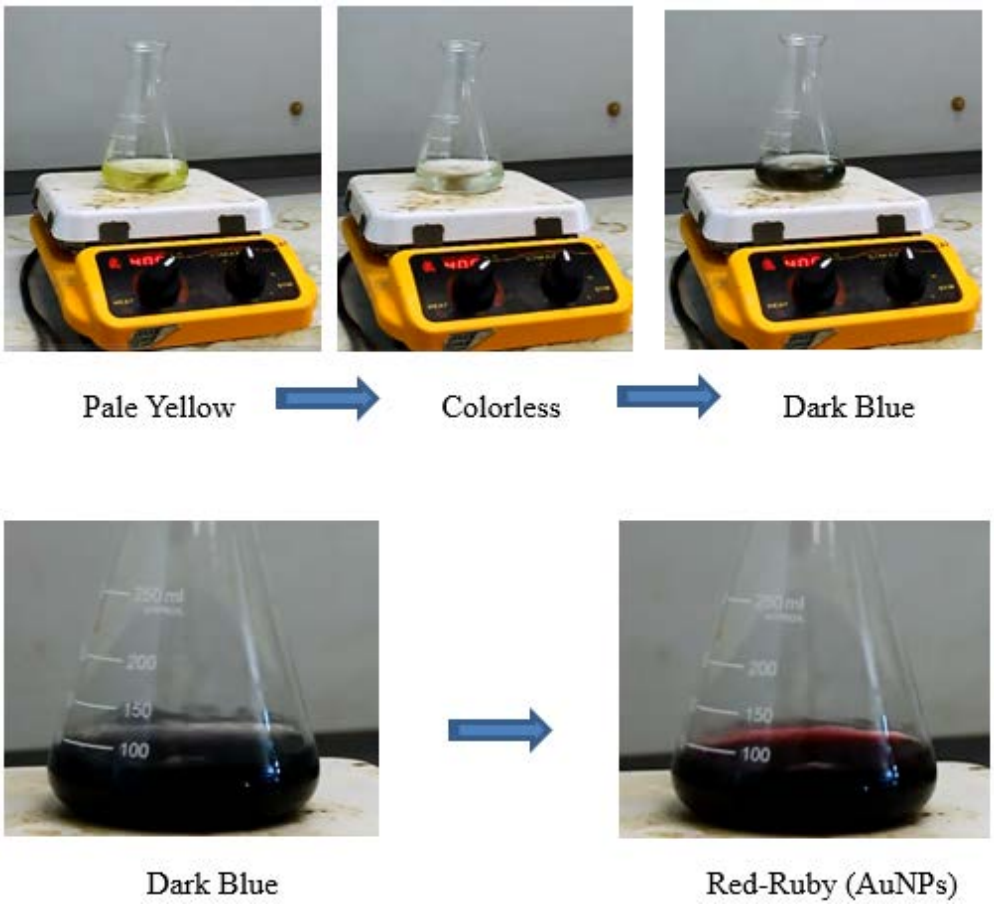
Description	Amount
0.1 M $HAuCl_4$	1 mL
38.8 mM sodium citrate	10 mL
10% TCA	10 mL
SPE elution solvent	20 mL
Chloroform	5 mL

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 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 minutes. (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 minutes.
5. Label the centrifuge tubes according to the data sheet.

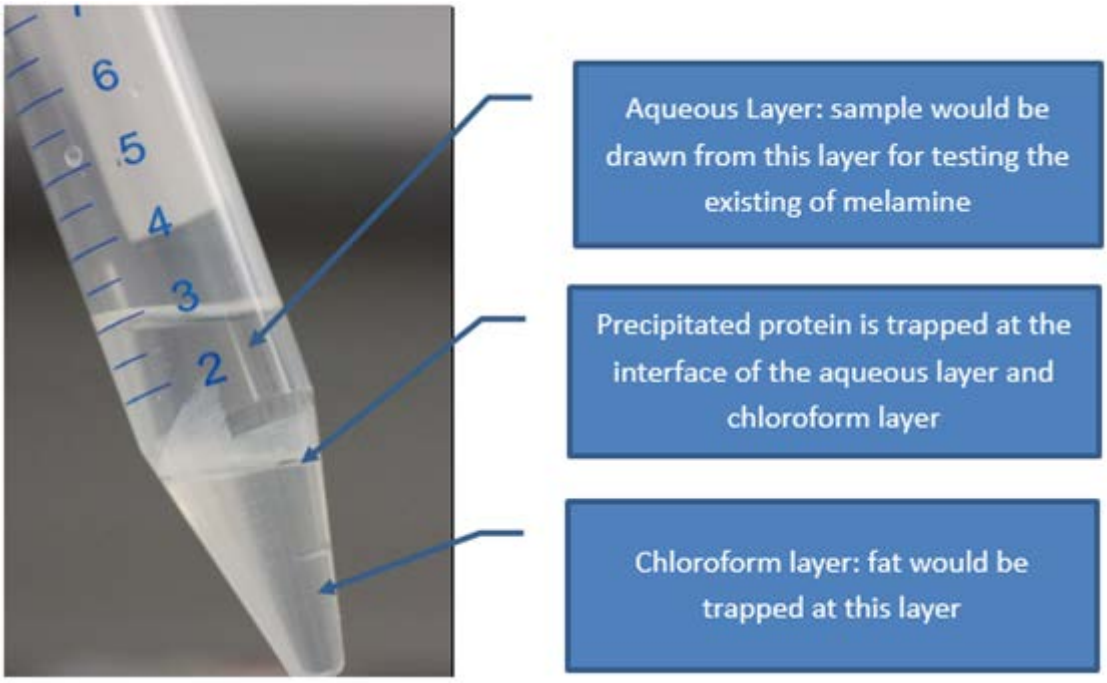
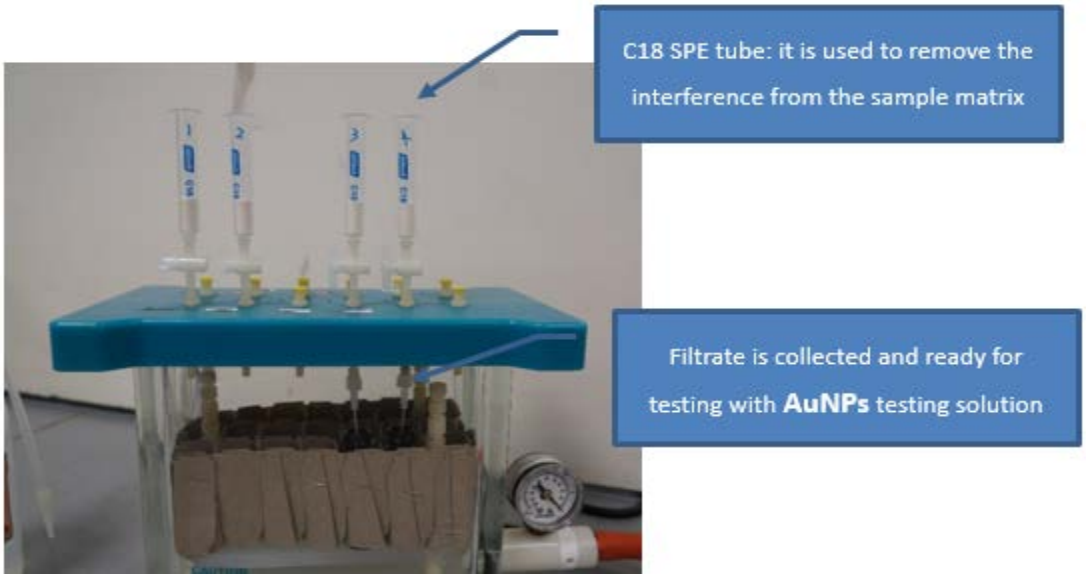


Fig. 3. After the centrifuge treatment, protein is precipitated out from the aqueous solution.

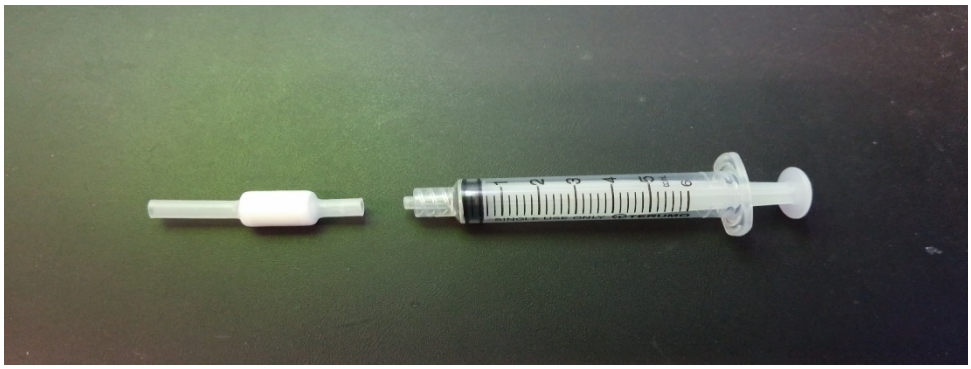
c. To clean up the supernatant using C18 SPE tube

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

Results and Discussion

If melamine does exist in the sample, varying from several parts-per-billion (ppb) to several hundred ppm, the screening with AuNPs will give a very fast preliminary guidance on whether further analytical testing is required.

Quantitative determination of the melamine content in sample using AuNPs can be achieved with the use of UV-visible spectrophotometry, as shown in Fig. 5 and 6.

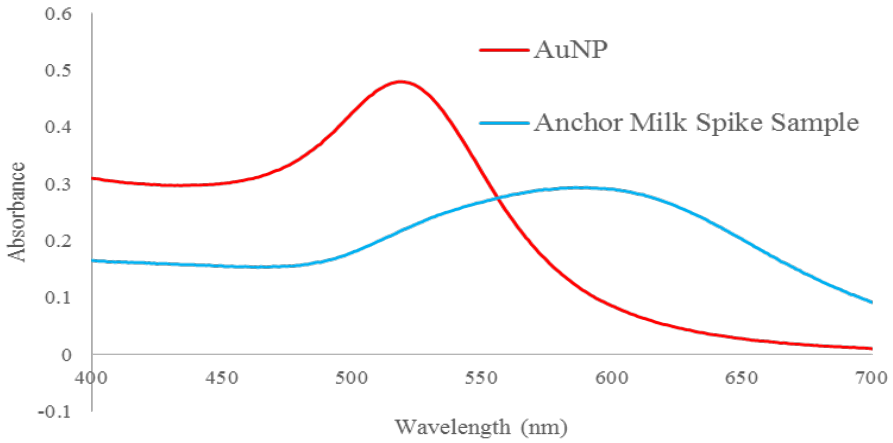


Fig. 5. UV-Vis Spectra of AuNP solution and AuNP with melamine spiked milk sample. The maximum absorption peak shifts to the longer wavelength region indicating the formation of gold nanoparticles clusters induced by melamine.

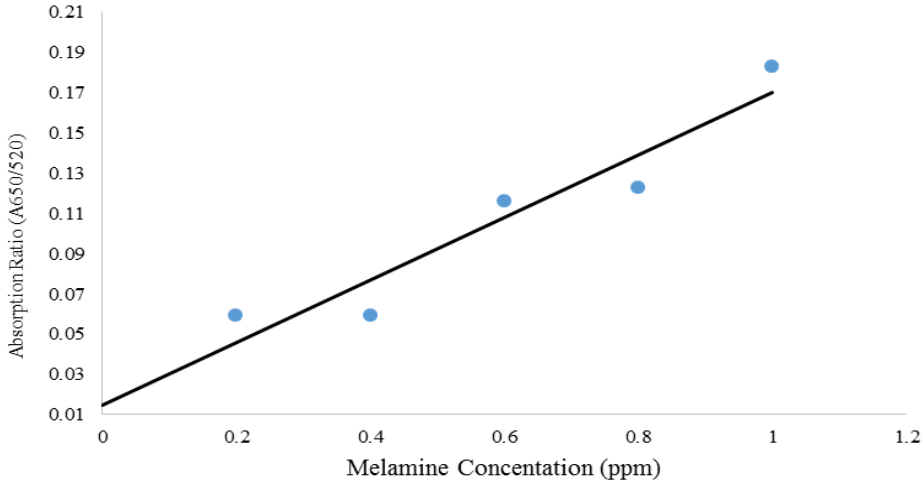


Fig. 6. Plot of $A_{650/520}$ for melamine concentrations of 0 – 1 ppm with AuNP.

In addition, high performance liquid chromatography coupled with mass spectrometer (HPLC-MS) could be employed for an accurate qualitative and quantitative analysis.

Conclusion

Topics of colourimetry and nanotechnology are included in HKDSE Chemistry and Physics Elective topics. Through this 2-hour lab session, students should appreciate

the development of a fast, low-cost, simple yet sensitive technique which can be adopted for daily life applications. Despite of the simplicity of the screening test for melamine using gold nanoparticles, without any advanced instrumentation, the sensitivity of melamine detection is comparable with that of high-end instrumentation (e.g., HPLC-MS) in ppm level. Meanwhile, students should have a better picture of a typical testing protocol of real samples which involves the sample-pretreatment and instrumental chemical analysis.

Questions & Answers

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

   Pale yellow → colourless → dark blue → red ruby

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

   It has two roles, (i) as a reducing agent to reduce the Gold (III) to Gold atom; and (ii) act as a stabilizer during the formation of AuNPs (to prevent agglomeration and further growth of size).

3. What is the function of C18 SPE tube?

   To remove the matrix interference (non-polar substances) from the milk samples.

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

   If yes, it offers a setup of low-cost that could achieve a fast screening purpose prior to use of any more advanced analytical instrument like LC/MS.

   If no, the formation of hydrogen bonding between melamine and AuNPs is not specific that might be interfered by complex sample matrix.

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 into 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 tube for further analysis. The changed colour and/or the presence of particles can bemore 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 treating menstrual 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].

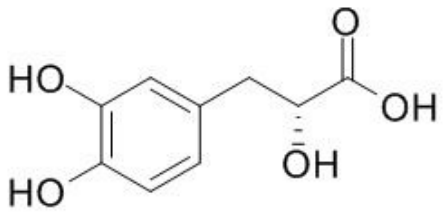


Fig. 1. Chemical Structure of Danshensu.

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, so 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.

The application of using iron (III) chloride solution for detecting the presence of phenolic functional groups in Danshen is an example to illustrate the contribution of analytical chemistry in the analysis of food and drugs which is an important element of the elective subject analytical chemistry of the current Senior Secondary Chemistry Curriculum. Apart from this, this experiment can broaden the knowledge of HKDSE students in typical reactions of various functional groups of organic compounds covered in the current curriculum. It also aims at arousing the interest of students to further explore the application of modern instruments, like HPLC, in chemical analysis in daily life.

Since the use of TCM in treatment of illness or enhancement of overall health is widely adopted in the East, the demand for TCM grows quickly. However, TCM are mostly provided in dried form and the morphologies of some other TCMs are very similar. These other TCMs with similar appearance may have entirely different therapeutic functions. Therefore the identification of TCM becomes very important.

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 [120-80-9]
- Iron (III) chloride [7705-08-0]
- 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.

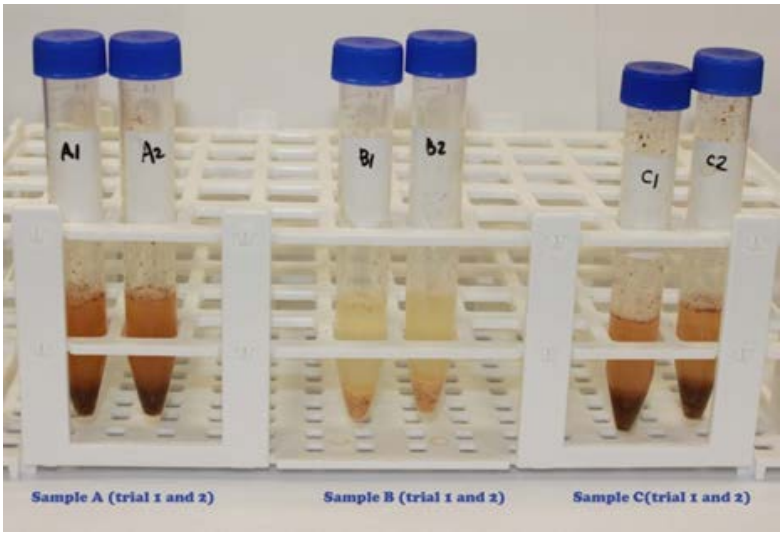


Fig. 3. Sample A: (Danshen from source A) acts as positive control,
Sample B: (Ginseng from source A) acts as negative control,
Sample C: (Danshen from source B) acts as sample.

Both Danshen (positive control) and Ginseng (negative control) samples (i.e. Samples A & B) can be purchased from local reputable Chinese medicine pharmacy shops. Danshen Sample C can be purchased from ordinary Chinese medicine pharmacy shops. These are ground into fine powder and ready for use.

4. Heat the mixture in a water bath for 15 min and then cool down.

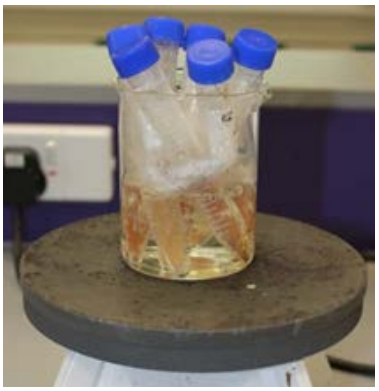


Fig. 4. Set up for a hot water bath for extraction.

5. Use centrifuge to spin down the solid sample.

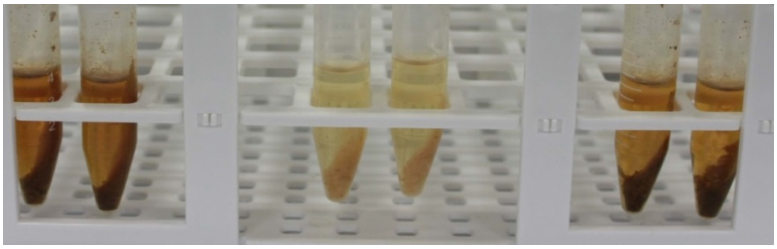


Fig. 5. After centrifugation, the suspended solid is gathered to the interior of the centrifuge tubes.

6. Transfer 1 mL of the supernatant to another empty new test tube.

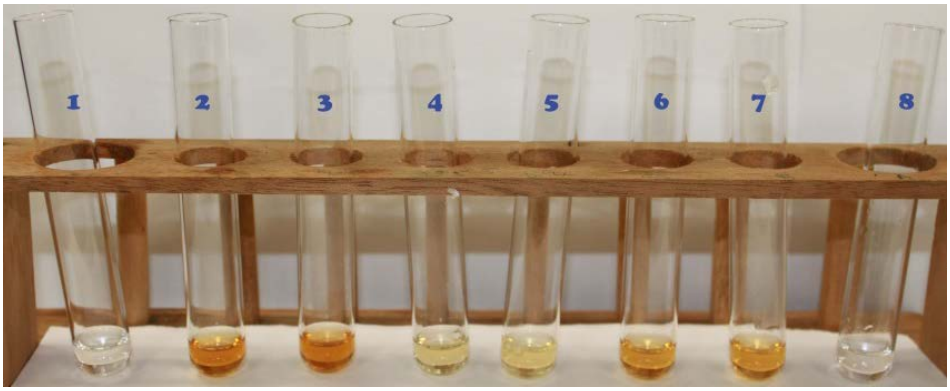


Fig. 6. Tube 1: Blank (water); Tube 2: positive control (trial 1); Tube 3: positive control (trial 2); Tube 4: negative control (trial 1); Tube 5: negative control (trial 2); Tube 6: sample (trial 1); Tube 7: sample (trial 2); Tube 8: 1,2-dihydroxybenzene (chemical standard).

7. Add 0.1 mL of $FeCl_3$ indicator solution to each test tube.

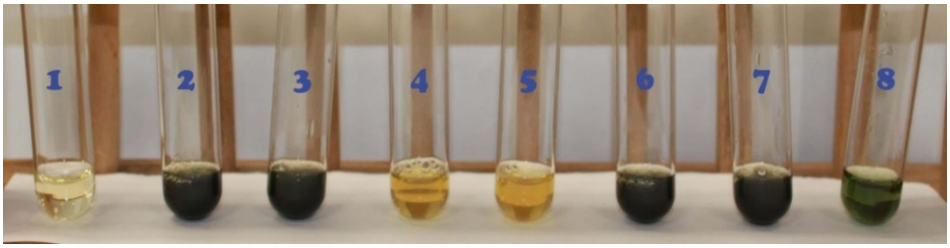


Fig. 7. Tube 1: Blank (water); Tube 2: positive control (trial 1); Tube 3: positive control (trial 2); Tube 4: negative control (trial 1); Tube 5: negative control (trial 2); Tube 6: sample (trial 1); Tube 7: sample (trial 2); Tube 8: 1,2-dihydroxybenzene (chemical standard).

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

Results and Discussion

Morphological appearance

	Sample A	**Sample B**	**Sample C**
Shape	Small size with root liked shape	Pale yellow pieces	Large pieces of root liked shaped
Colour	Dark red	Pale yellow	red
Colour of powder form	Pale red	Light brown	Pale red

Colour of the extracted solution

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

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

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

The water-soluble chemicals in Danshen are selectively extracted by water using a hot water bath. By reacting with $FeCl_3$ solution, it gives a characteristic green complex. In this experiment, a water control is used to avoid risk of contamination. Test tube 8 is a chemical standard for reference. The generation of green colour provides a reference colour for positive experimental observation.

Conclusion

A facile test-tube scale experiment is used to differentiate Danshen from other similar TCMs. The laboratory activity involves two steps: (1) extraction of the active ingredient(s) from a real-life TCM sample; and (2) confirmation of the presence of the essential component in the herb by a simple qualitative analysis method. This analytical approach can be extended to other TCMs.

Questions and Answers

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

   Breakdown of solid sample in fine powder results in increased surface area and thus the extraction efficiency

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

   By refluxing the sample with longer time

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

   Make sure the colour change is due to the presence of the phenolic functionalities of the chemicals

4. Why do we need to duplicate the experiment?

   Increase the accuracy of the experiment

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

   Danshen contains a lot of other water-soluble chemical fractions. These chemicals will become interference and provide slightly different colour to the one produced from chemical standards

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)

   Yes, the Chinese Medicine division of the Department of Health launched a project in 2002 for developing standards of commonly used TCM materials in Hong Kong. The Hong Kong Chinese Materia Medica Standards (HKCMMS) covering 299 TCM materials have been published so far.

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[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.

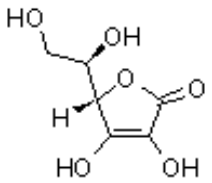
Vitamin C plays an important role in immune function. Insufficient vitamin C causes muscles weakness, swollen and bleeding of gums, loss of teeth and bleeding under the skin as well as tiredness and depression.

There are many methods that can be applied to determine vitamin C in fruits or a vitamin supplement. Herein iodometric titration is applied to determine the amount of vitamin C in either fresh fruits or dietary supplement in tablet forms. 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

In the current Senior Secondary Chemistry Curriculum, the volumetric analysis of titration has been fully covered. It is also required to equip the students with the knowledge of redox chemistry. The principle and the content of this experiment could confer teachers and students with the importance of analytical technique of redox titration and quantifying the vitamin C in real-life samples of fruits using appropriate test.

The quantitative determination of vitamin C in food is beneficial to the public health. The determination is, therefore, one of the important aspects in food analysis of the testing and certification sector. Standard methods of AOAC Official Method 967.21 and 967.22 are widely used in local commercial testing laboratories.

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 [7681-11-0]
- Potassium iodate [7758-05-6]
- Sulfuric acid [7664-93-9]
- L-ascorbic acid (Vitamin C) [50-81-7]
- Starch [9005-25-8]

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

With a fresh orange

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).

With a Vitamin C tablet

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

Fig. 4. End point of the titration

Remarks: Due to the limited time of two laboratory sessions, it is recommended that the standard solution of vitamin C and iodine solution could be prepared in advance by technicians of the school.

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

Results and Discussion

Observation

1. At the end point of the titration of standardization of iodine solution, the colour of the solution changes from colourless to blue.
2. At the end point of the titration of determination of vitamin C content, the colour of the solution changes from pale yellow to dark yellow.

Data and data treatment

Weight of potassium iodide = 5.08 g

Weight of potassium iodate = 0.2720 g

Weight of pure vitamin C = 0.2511 g

Total volume of the freshly squeezed juice = 90 mL

Standardization of iodine solution

	1st trial	2nd trial	3rd trial
Initial burette reading (mL)	0.00	14.50	28.80
Final burette reading (mL)	14.50	28.80	43.10
Volume of iodine solution used (mL)	14.50	14.30	14.30

$$2\ IO_3^- + 10\ I^- + 12\ H^+ \longrightarrow 6\ I_2 + 6\ H_2O$$

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

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

$\therefore$ Mole ratio of $I_2 : C_6H_8O_6 = 1 : 1$

The number of moles of ascorbic acid used in each trial = 1.43×10^{-4} mol

The average volume of iodine solution used = 14.37 mL

$\therefore$ The real concentration of the iodine solution = 0.00995 M

Note:

- [number of mole of $C_6H_8O_6$ (mol)] = [mass of $C_6H_8O_6$ (g)] / [molar mass of $C_6H_8O_6$ (g/mol)]
- molar mass of $C_6H_8O_6$ = 176.12 g/mol
- [concentration of I_2 (M)] = [number of mole of $C_6H_8O_6$ (mol)] / [volume (dm^3)]

Iodine solution must be standardized against L-ascorbic acid of known molarity prior to the use as titrant. The formation of the active triiodide ion involves an equilibrium reaction between iodine and iodide. The concentration of triiodide content in a solution would vary from the expected value. If standardization is not performed, there will be errors in the measurement.

Determination of vitamin C content in tablet by titration

	1st trial	2nd trial	3rd trial
Initial burette reading (mL)	0.00	13.90	27.50
Final burette reading (mL)	13.90	27.50	41.20
Volume of iodine solution used (mL)	13.90	13.60	13.70

Note:

- [number of mole of iodine (mol)] = [concentration of iodine (M)] x [volume (dm^3)]
- As shown above, the real concentration of the iodine solution = 0.00995 M
- molar mass of $C_6H_8O_6$ = 176.12 g/mol
- [mass of $C_6H_8O_6$ (g)] = [number of mole of $C_6H_8O_6$ (mol)] x [molar mass of $C_6H_8O_6$ (g/mol)]

The average volume of iodine solution used = 13.73 mL

The average number of moles of iodine used = 1.36×10^{-4} mol

The average number of moles of vitamin C = 1.36×10^{-4} mol

The mass of vitamin C content determined (mg) = 24.06 mg

$\therefore$ The vitamin C content of tablet (mg) = 962.4 mg per tablet

Titration of fresh fruit juice

	1st trial	2nd trial	3rd trial
Initial burette reading (mL)	0.00	11.20	27.40
Final burette reading (mL)	8.60	19.60	36.10
Volume of iodine solution used (mL)	8.60	8.40	8.70

The average volume of iodine solution used = 8.57 mL

The average number of moles of iodine used = 8.52×10^{-5} mol

The average number of moles of vitamin C = 8.52×10^{-5} mol

The mass of vitamin C content (mg) = 15.01 mg

$\therefore$ The vitamin C content of fresh fruit juice (mg) = 54.04 mg

Possible measurement using advanced instrumentation

According to AOAC Official Method 967.21, the vitamin C content of food can be determined by 2,6-dichloroindophenol titrimetric method[1]. The method is applicable to all kinds of fruits, except the juices with colours. Besides, vitamin C can be determined by spectroscopy method (AOAC 967.22)[2], in which the ascorbic acid is converted to dehydroascorbic acid through oxidation. The dehydroascorbic acid is then reacted with ortho-phenylenediamine to form a fluorophor in solution. After that, the intensity of the fluorescence is measured and compared to the fluorescence of a standard. On the other hand, vitamin C is allowed to be determined by High Performance Liquid Chromatography method coupled to UV detection[3]. The Government Laboratory has developed the HPLC-UV method to determine the vitamin content in milk powder [4]. However, both spectroscopic and chromatographic methods require specific extraction methods for sample pretreatment.

Conclusion

A redox titration is a type of titration based on a redox reaction between the analyte and the titrant. The technique can be applied for quantitative determination of vitamin C in food without the need of repetitive sample pretreatments. Through the experiment, students can acquire the concept of redox chemistry in quantitative analysis. The data obtained in the experiment can be checked with the regulations in Hong Kong, which helps to develop student awareness of nutrients in food and also interest in food analysis.

Questions and Answers

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

   The nutrient label indicates that the tablet contains 1000 ppm vitamin C, which shows a good agreement with the experimental result of 962.4 ppm.

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

   The suggested daily intake of vitamin C for a healthy adult is about 90 mg for men, and 75 mg for women. In this experiment, an orange is found to contain 54 mg of vitamin C, which is shown to be a good source.

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

   2,6-dichloroindophenol titrimetric method

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?

   Vitamin C is required to be identified on the nutrition labels for infant formula and when a nutrition claim is made on vitamin C on the food product.

   There are commercial testing laboratories in Hong Kong that can test for all the items on the nutrition label.

References

[1] AOAC international: *Official Methods of Analysis of AOAC (2005)*, 18th Edition., ed. Horwitz W., Gaithersburg: MD, Method 967.21.

[2] AOAC international: *Official Methods of Analysis of AOAC (2005)*, 18th Edition., ed. Horwitz W., Gaithersburg: MD, Method 967.22.

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