Conceptual Chemistry
Glow In The Forensic Investigations

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Conceptual Chemistry

Conceptual Chemistry is a graduate course designed for grade school and middle school teachers to assist in their understanding of chemistry and to provide concrete ideas that they can take back to their classrooms to teach their students.

Conceptual Chemistry

Participants in this course receive:

- Free tuition and five graduate credit hours from the College of Education of Kent State University. *($2,375 value)*
- Over $850 worth of materials and supplies to take back to the classroom.

Conceptual Chemistry

Support for *Conceptual Chemistry* and the development/production of this material was provided by a grant under the federally funded *Improving Teacher Quality State Grants Program*, administered by the Ohio Board of Regents.

Spectroscopy

Spectroscopy
The investigation into the nature of matter using electromagnetic radiation.

Most Common Types
Infrared, ultraviolet, visible, NMR, X-ray and microwave.

Activity Objectives

Key concepts:

- Molecular Spectroscopy
- Electromagnetic Radiation
- Chemical Changes
- Physical Changes
- Acid/Base Chemistry
- Quantitative and Qualitative Analyses
- Having fun with science!

Spectroscopic Analysis

Purpose
To determine the identity of a“ heinous evil-doer” responsible for attempting to poison our project patron and all around good guy, Dr. R. U., using semi-quantitative spectroscopy.

Activity Objectives

Agent code-named E. Snowed-in of the ASN, a secret organization, just finished eating a lunch and is now waiting to meet with Dr. R. U. We suspect that Agent Snowed-in may be attempting to poison Dr. R. U., however, we are unsure if this is a real threat or just a ruse. We currently lack the evidence necessary to stop this evil deed and make an arrest. Your job, if you choose to accept it, is to determine the true identity of Agent Snowed-in and obtain evidence of this alleged plot.

Crime Scene Set-Up

The “crime scene” consisted of the following:

1 clear plastic cup filled with Mt. Dew that was contaminated with the extract* from a Sharpie brand, yellow highlighter to simulate poisoning with automobile anti-freeze (ethylene glycol). The clear plastic cup had a fingerprint mark left as a result of wetting a finger with a small amount of vitamin A and then carefully applying the print to the cup with a light touch.

*The highlighter extract was prepared by opening a new Sharpie highlighter and rinsing the ink reservoir with 100 ml distilled water.

1 Styrofoam cup filled with tonic water.

1 bowl with the residue from Lipton brand “Extra Noodle” chicken soup. The soup was prepared as per the manufacturers instructions and allowed to sit in the bowls overnight. The soup was then discarded and the bowls were permitted to dry. The bowl was also marked with Tide brand laundry detergent providing a fluorescent marking.

Each of the crime scene cups was labeled with a yellow highlighter. The labels were “yours” on the clear plastic cup and “mine” on the Styrofoam cup. The Styrofoam cup was rinsed with water to conceal the label.

**Important note: The yellow color of the highlighter was easily removed from the cups by rinsing with water. However, the fluorescent dye component of the highlighter was left behind on porous material such as Styrofoam or human skin. This made for a very effective method of labeling materials/people with “invisible” ink and identifying the “alleged criminal”. Although the highlighter pens are labeled as non-toxic by the manufacturer, caution should be used if applying the ink to human skin. Refer to the SDS provided by the pen manufacturer for more safety details.

Ultraviolet Light

UV-A
320-400 nm, lowest energy UV light, suspected of damaging skin (premature aging and wrinkling), and skin cancer.

UV-B
280-320 nm, affects DNA, eyes, immune system. UV radiation at 280 nm is 10^5 times more damaging than UV radiation at 320 nm.

UV-C
10 - 280 nm, (10 nm – 120 nm is *ionizing* EMR) very high energy, most harmful.

Ultraviolet Light

UV-A
320-400 nm, lowest energy UV light, suspected of damaging skin (premature aging and wrinkling), and skin cancer.

Fluorescence, Phosphorescence & Luminescence

Fluorescence
Emission of light ( ) as a result of a substance absorbing EMR of *shorter* wavelength.

Phosphorescence
Emission of light *long after* being initially irradiated by another light source. (Glow-in-the-dark)

Fluorescence, Phosphorescence & Luminescence

Chemo-luminescence
Low temperature emission of light as a result of a chemical reaction. The emission of light that does not derive energy from the temperature of the emitting body.

Fluorescence

Absorption of EMR followed by intermediate vibrational energy losses resulting in light emission in the visible region of the electromagnetic spectrum.

quinine

Fluorescent Molecules

quinine

pyranine

Riboflavin (vitamin B_2)

vitamin A (retinol)

Fluorescent Materials

Materials

- Liquids from crime scene.
- Other crime scene materials:
  - Cups, bottles, dust etc.

Fluorescence

Procedure

- Shine a UV light onto each substance at the crime scene.
- Identify fluorescent and phosphorescent materials.
- Make a list of the materials that luminesce.
- Distinguish between the types of luminescence. (fluorescence, phosphorescence or chemo-luminescence.)

Fluorescence

Results

- Colorless liquid – soda?
- Yellow liquid – soda? Mt. Dew?
- Bowl residue
- Powder
- Fingerprint on cup
- Markings

Reference Materials

Common Fluorescent Materials

- Soda Pop
  - Tonic water
  - Mt. Dew?
- High-lighter markers
- Antifreeze
- Liquid detergents & soaps
- Vitamins (A and B complex, especially B_2)
- ~~Body fluids – urine & semen~~

Reference Materials

Demonstrations:

- Soda Pop
  - Tonic water
  - Mt. Dew
- High-lighter markers
- Antifreeze
- Liquid detergents & soaps
- Vitamins (A and B complex, especially B_2)

Quenching

Quenching
The elimination of fluorescence by addition of a chemical agent that interferes with the energy emission process.

pyranine

Spectroscopic Analysis

Purpose
Attempt to *quench* the fluorescent activity of the liquids at the "crime scene".

Quinine – Quenches upon addition of aqueous salt (NaCl) solution.

Vitamin B_2 – Quenches upon addition of baking soda or salt solution ($AgNO_3$ etc.).

Pyranine – Quenches upon addition of strong acid (pH 3 or lower).

Spectroscopic Analysis

Materials

- 2 test tubes
- salt (NaCl) water solution
- 1.0 ml graduate plastic pipet
- UV light source

Spectroscopic Analysis

Procedure

- Place ~5 mL of crime scene liquid into the test tube.
- Add saturated NaCl solution in 0.5 mL portions up to 3 mL.
- Observe changes.

Spectroscopic Analysis

Materials

- 24 well plate
- 1.0 M HCl solution
- 1.0 M NaOH solution
- 0.1 M silver nitrate ($AgNO_3$) solution
- 1.0 ml graduate plastic pipet
- UV light source

Spectroscopic Analysis

Procedure

- Place 1-2 drops of each highlighter marker extract into each of the first five wells A-1 through A-5.
- Add ~1 ml water into each of the wells containing highlighter.
- Add 5 drops 1.0 M HCl
- Observe changes.

Spectroscopic Analysis

Procedure

- Add 5 drops 1.0 M NaOH to wells A-1 through A-5.
- Observe changes.

Spectroscopic Analysis

Procedure

- Wells C-1 and C-3 contain vitamin B-2 extract.
- To well C-1 only:
  - Add 5 drops 1.0 M HCl
  - Observe changes.
  - Add 5 drops 1.0 M NaOH to
  - Observe changes.

Spectroscopic Analysis

Procedure

- Wells C-1 and C-3 contain vitamin B-2 extract.
- To well C-3 only:
  - Add 1-2 drops 0.1 M $AgNO_3$
  - Observe changes.

Spectroscopic Analysis

Purpose

To determine the concentration of *quinine* in tonic water using qualitative spectroscopy.

quinine

History of Tonic Water

Quinine is an anti-pyretic, anti-inflammatory, analgesic, anti-malarial bitter tasting drug and has been used for hundreds of years to treat malaria symptoms.

quinine

Fluorescence Spectroscopy

Fluorescence Spectroscopy

- Very expensive
- May be simulated with a smart phone and a free, open-source software application, *ImageJ*.
- *ImageJ* is an open-source, Java-based program developed at NIH and allows for various analyses of electronic images.

Spectroscopic Analysis

- concentration of a solution is directly proportional to its absorption/fluorescence of electromagnetic radiation.

$$A = \varepsilon bc$$

Where:
A = absorbance/fluorescence
ε = molar extinction coefficient
b = sample cell path length
c = sample concentration

Molecular Fluorescence

Fluorescence values were obtained from the digital images by measuring the RGB (red, green, blue) values (on a scale of 0−255 intensity) of the samples using *ImageJ.*

$$F = \log \frac{I_f}{I_0}$$

Where:
F = fluorescence
I_f = Intensity of sample (green channel)
I_0 = Intensity of blank (green channel)

Spectroscopic Analysis

Materials

12 ml tonic water
Distilled water
6 glass test tubes
1.0 ml graduate plastic pipet
UV light source

Tonic water is used as purchased and assumed to contain 60 mg/L quinine

Spectroscopic Analysis

Procedure

1. Obtain a 15 ml plastic conical tube containing tonic water *(12 ml "stock" solution).*
2. Use a plastic pipette to withdraw 8.0 ml of tonic water from the conical tube containing the "stock" solution and place it into the *first* test tube. *(This leaves 4.0 ml tonic water solution in the plastic tube.)*

Spectroscopic Analysis

Procedure (cont.)

3. Add 8.0 ml water to the plastic conical tube, replace the cap and mix thoroughly. *(This gives 12 ml of solution again!)*
4. Withdraw 8.0 ml of the diluted tonic water from the conical tube and place it into the *second* test tube. *(This leaves 4.0 ml drink mix in the plastic tube again.)*

Spectroscopic Analysis

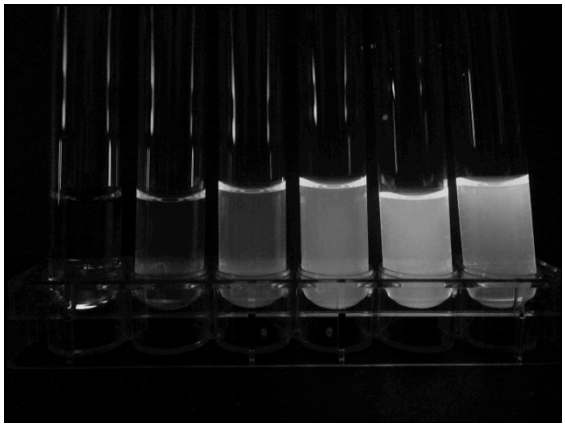
Procedure (cont.)

5. Add 8.0 ml water to the plastic conical tube, replace the cap and mix thoroughly.
6. Continue this dilution process three more times until *five* glass test tubes are filled.
7. Shine a UV light onto the test tubes being careful not to expose yourself or others to the light. Note the variable brightness of the tubes with respect to concentration.

Spectroscopic Analysis

Procedure (cont.)

8. Place 8.0 ml of the *"unknown"* sample from the crime scene into the *sixth* test tube.
9. Compare the *"unknown*" sample to the five standards to determine the dilution ratio of the unknown sample.
   dissolved into 1,000 ml solution).



Spectroscopic Analysis

Spectroscopic Analysis

Dilution Math

Tonic water is regulated and may only contain up to 83 ppm of quinine. Most commercial tonic water ranges from 25 -60 ppm.

$$60 \text{ ppm} \times \frac{4 \text{ ml}}{12 \text{ ml}} = 20 \text{ ppm}$$

Tube #1	Tube #2	Tube #3	Tube #4	Tube #5
60 ppm	20 ppm	6.7 ppm	2.2 ppm	0.70 ppm

Spectroscopic Analysis

Concentration mg/L	Fluorescence	Green Channel
60.0	1.52	187
20.0	1.39	139
6.7	1.18	86
2.2	0.78	34
0.0	0.00	5.7
unknown	**1.17**	**135**

"Unknown" was prepared to match "Tube #3"

Spectroscopic Analysis

Dilution Math

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$$60 \text{ ppm} \times \frac{4 \text{ ml}}{12 \text{ ml}} = 20 \text{ ppm}$$

Tube #1	Tube #2	Tube #3	Tube #4	Tube #5
60 ppm	20 ppm	6.7 ppm	2.2 ppm	0.70 ppm

"Unknown" was prepared to match "Tube #3"

Conclusions

Suspect	Marker/Note	Soup	Drink	Fingerprint	Dust
Fenk	**X**	**X**	**X**	**X**	**X**
Khourey-Bowers		---	---	---	X
Gerbig		---	X	---	X
Bille		X	---	---	
O'Connor		X	---	---	

Conclusions

- A new method for determining semi-quantitative fluorescence using a digital camera and ImageJ software was presented.
- UV light was used to semi-quantitatively determine the concentration of unknown samples.
- The evil-deed doer was brought to justice.