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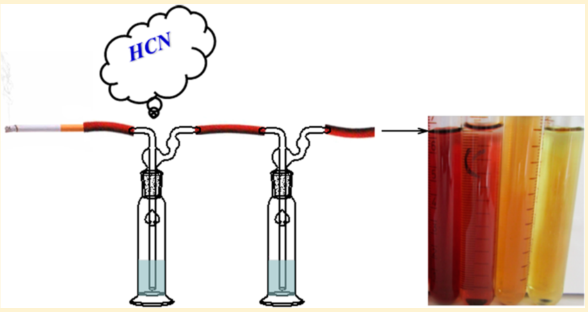
Visualizing Smoking Hazard: A Simple Spectrophotometric Determination of Hydrogen Cyanide in Cigarette Smoke and Filters

Andriana R. Surleva† and Gabi Drochioiu*

Faculty of Chemistry, Alexandru Ioan Cuza University of Iasi, Iasi 700506, Romania

Supporting Information

ABSTRACT: An activity for hydrogen cyanide determination in cigarette smoke is described. The procedure is based on the absorption of hydrogen cyanide (HCN) from cigarette smoke in a 2% sodium carbonate solution followed by its reaction with ninhydrin and a color intensity measurement at 485 nm. To quantify HCN in cigarette smoke, the previously determined molar absorptivity is used. A naked-eye experiment is proposed to demonstrate the presence of HCN in cigarette smoke and to visualize smoking hazard.



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Cyanide has been known as a serious poison since ancient times. Plants containing cyanide derivatives, such as bitter almonds, cherry laurel leaves, peach pits, and cassava, have been used as lethal poisons. Peach pits that had been used in judicial executions by the ancient Egyptians are on display at the Louvre Museum, Paris. The Romans used cherry laurel leaves as a method of execution (also known as "the cherry death"). Cyanide was first produced expressly for the purpose of killing during World War I. During World War II, the Nazis used HCN to exterminate people in concentration camps. Cyanide was detected in the walls of crematoria almost 50 years later. Cyanide has been the typical agent used in "gas chambers" for judicial executions; usually, a cyanide salt is dropped into an acid to produce HCN.[1]

However, despite having been widely discussed as a very toxic compound, few people realize that they are being exposed to hydrogen cyanide from cigarette smoke. The HCN toxicity from environmental tobacco or fire smoke inhalation has been recently discussed.[2,3] It is one of the 44 harmful substances in cigarette smoke that inhibits several respiratory enzymes, and is a major cilia toxic agent, which causes damage to certain body organs.[4] The amount of cyanide present in cigarette smoke could have a deleterious effect on human health, particularly the central nervous system. The HCN levels in cigarette smoke have been reported to range from 10 to 400 μg/cigarette for inhaled smoke, and from 0.006 to 0.27 μg/cigarette for side stream (secondhand) cigarette smoke.[5] Hydrogen cyanide in cigarette smoke is primarily produced by the conversion of protein, amino acid, and nitrate. Upon inhalation, HCN quickly enters the bloodstream. HCN produces toxic effects at levels of 40 μM or more.[6] Cyanide in blood is either loose in plasma or bound to ferric hemoglobin. Some of the cyanide is naturally converted to thiocyanate and is subsequently excreted through urine.[7]

The extreme toxicity of cyanide compounds continues to elicit intensive research on selective and sensitive methods for cyanide detection.[1,8] Hydrogen cyanide in cigarette smoke was determined using capillary gas chromatography coupled with a microelectron capture detector[9] or ion chromatography with pulsed amperometric detection.[10] Both methods use automated standard smoking machines for sampling. An easily built smoking machine to be used by undergraduate students for the determination of total particulate matter and nicotine in tobacco smoke has been recently proposed.[11] The papers regarding cigarette smoking hazards[12] published in this *Journal* have been mainly focused on NO_x,[13] trace metals,[14] carbon dioxide, carbon monoxide and methane,[15] hydrogen sulfide,[16] formaldehyde[17] determination, or demonstration of the chemical process of smoking and identification of some of the principal smoke components.[18−20] However, the HCN determination and its toxicity have not been specifically discussed.

We have noticed that when discussing HCN as a poison, all students seemed to be shocked to learn of its usage as a killing agent. However, when discussing cigarette smoking and the risks caused by HCN for smokers, students have reacted with a bit of doubt. Here, we propose an experiment to demonstrate the presence of HCN in cigarette smoke and to persuade young people to reconsider everyday exposure to HCN from cigarette smoke. The smoking hazards visualization is based on the color reaction between ninhydrin and cyanide ions in alkaline medium.[21−23] The described activity can also be used for HCN quantification in cigarette smoke or filter, using previously determined molar absorptivity, in order to avoid the use of hazardous potassium cyanide standards by students.

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In contrast to the reported chromogenic reagents for cyanide determination in literature, the ninhydrin−cyanide reaction is highly sensitive, simple, and fast.[21,22] The reagents used are nonhazardous, inexpensive, and readily available.

■ AUDIENCE

The experiment could be integrated in the curriculum of chemistry or biology as a "naked eye" classroom activity or demonstration to prove the presence of HCN in smoke, and to motivate discussions on smoking hazards. In the undergraduate biochemistry or analytical chemistry curriculum, it is applicable for obtaining quantitative data using previously determined molar absorptivity. For example, the experiment was used in a biochemistry course to obtain quantitative data for hydrogen cyanide in used cigarette filters or in cigarette smoke by spectrophotometric measurements. Students were additionally motivated by working on a "brand new science": the ninhydrin assay was first reported in 2002[21,22] but was improved recently.[23] By participating in this activity, students learn about the presence of highly noxious hydrogen cyanide in cigarette smoke and how to use a simple technique to detect it. Students learn how to build a simple smoking device using widely available lab glassware. They observe how the acidity of the medium influences the ionization state and the color of a solution containing the same chromogenic reagent.

■ ACTIVITY

Sampling

HCN in cigarette smoke is collected by a solution-absorbing method (Figure 1). A simple apparatus is built for cigarette

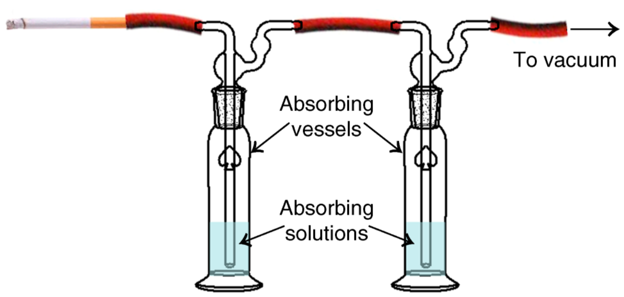

Figure 1. Sampling apparatus for absorption of HCN from cigarette smoke.

smoke sampling (set up the apparatus in a fume hood or well-ventilated space). Cigarette smoke is pumped through two absorbing vessels in series, connected to a water vacuum pump, each one containing 20.0 mL of 2% Na_2CO_3 solution. The two absorbing solutions are mixed; aliquots of 1 mL in volume are taken for analysis. For accurate quantitative spectrophotometric measurements, the absorbing solutions have to be purged with nitrogen before adding the ninhydrin reagent.

The HCN trapped by a cigarette filter is extracted in 10 mL of 2% Na_2CO_3 for 15 min that is being agitated by a magnetic stirrer. Aliquots of 20 μL of obtained solution are added to test tubes containing 1 mL of 2% Na_2CO_3. If the obtained extract is not clear enough for spectrophotometric measurements, the solution has to be centrifuged for 5 min at 10,000 rpm, or filtered using fine filter paper.

Preparation of Ninhydrin Reagent

Ninhydrin solution has to be freshly prepared by dissolving 250 mg of ninhydrin in 50 mL of 2% Na_2CO_3 and purged with nitrogen for 15 min. All solutions are prepared using distilled water. For school demonstrations, the reagent can be prepared in the lab by the instructor in small portions (5 mL) and stored in dark bottles. In the high school classroom, students should use a newly opened bottle for the qualitative or quantitative analysis of each experiment. The ninhydrin solution purged with nitrogen can be stored in a tightly closed dark bottle for a week. For an undergraduate class experiment, the students should prepare fresh ninhydrin solution for quantitative experiments.

Qualitative Analysis (Naked Eye Experiment)

Add 0.5 mL of ninhydrin reagent to the 1 mL absorbing solution sample and observe the color changing. The solution color turns red if cyanide ions are present in the solution. Add some drops of NaOH solution (1 M); the color turns blue. Discussions on chromophore groups and different colors of mono- or double-ionized CN−ninhydrin complex would be appropriate among undergraduate students.[24] The spectra of ninhydrin in sodium carbonate solution, before and after adding KCN as well as after adding NaOH, are presented in Figure 2.

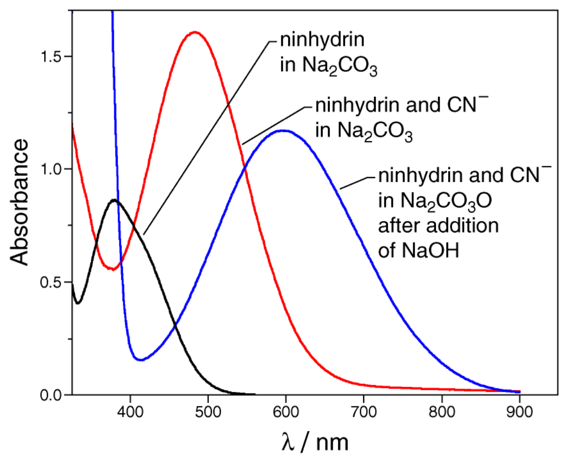

Figure 2. Spectra of ninhydrin in Na_2CO_3 solution (black line), ninhydrin in Na_2CO_3 solution after addition of the absorbing solution containing HCN (red line), and ninhydrin and absorbing solution in Na_2CO_3 solution after addition of NaOH (blue line).

Quantitative Analysis

Three aliquots of smoke-absorbing solution or filter extraction solution are used for a spectrophometric analysis, and 0.5 mL of ninhydrin solution is added to each test tube. The solutions in the test tubes are homogenized and left for 15 min in view of color development. The absorbance of each sample is measured at 485 nm against a blank containing 1 mL of 2% Na_2CO_3 and 0.5 mL of ninhydrin reagent (additional details can be found in the Supporting Information). The cyanide concentration in the absorbing solution is calculated using the Beer's law equation, and the preliminarily obtained value for molar absorptivity of the CN−ninhydrin complex in sodium carbonate is 1.4×10^5 L/(mol cm).[23] The mean value of concentration is used to calculate the HCN content in micrograms per cigarette or filter. For example, in the case of cigarettes purchased on the Romanian market, the mean content in cigarette (cig) smoke was 6.3 μg HCN/cig, and in a used cigarette filter, it was 55 μg HCN. The cyanide content in the absorbing solution decreased to 13 μg/L (corresponding to 1 μg HCN/cig) after one week in storage in a dark bottle, at room temperature with daily light illumination. Hence, the sample has to be analyzed in the same

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day. Additionally, the laboratory air was also analyzed using the same sampling procedure, and no cyanide was detected in the absorbing solution, assuring that the blank cyanide correction was not necessary.

DISCUSSION

Before beginning the activity, questions such as these may help prepare students for what they will see and do.

- Is cigarette smoking prevalent in your area (or city, region, or country, or among people of your age)?
- Are you familiar with any analytical methods for detection of hazardous compounds in the air?
- Do you know about a poison that could be used to kill a person, which is widely described in some crime novels or mysteries?

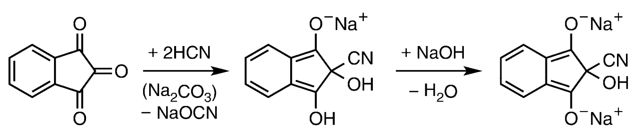
These example questions may help students consolidate what they saw (the qualitative results) and learned experimentally (the quantitative analysis) from this activity:

- Did you observe any change in color of the absorbing solution upon addition of the ninhydrin reagent? What does it mean if the color is yellow or red?
- How would you explain the color change from red to blue after addition of sodium hydroxide?
- This experiment used previously determined molar absorptivity data. What are the advantages and drawbacks of this method for determination of an unknown using spectrophotometry?

Some example answers are provided in the Supporting Information.

A chemical reaction between ninhydrin and hydrogen cyanide in the presence of sodium carbonate and sodium hydroxide is shown in Scheme 1. Such a reaction pathway may elucidate color changes of the absorbing solution on adding the ninhydrin reagent.[22,24]

Scheme 1. Ninhydrin Reaction with Hydrogen Cyanide Present in Cigarette Smoke



ninhydrin — red colored compound — blue colored compound

ASSOCIATED CONTENT

Supporting Information

Notes for instructor, hazards, and disposal. This material is available via the Internet at http://pubs.acs.org/.

AUTHOR INFORMATION

Corresponding Author

*G. Drochioiu. E-mail: gabidr@uaic.ro.

Present Address

†Department of Analytical Chemistry, University of Chemical Technology and Metallurgy, Sofia 1756, Bulgaria.

Notes

The authors declare no competing financial interest.

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