Demonstrating the Antioxidative Capacity of Substances with Lightsticks

Robert R. Wieczorek* and Katrin Sommer
Didaktik der Chemie, Ruhr-Universitat Bochum, 44780 Bochum, Germany
**robert.wieczorek@rub.de*

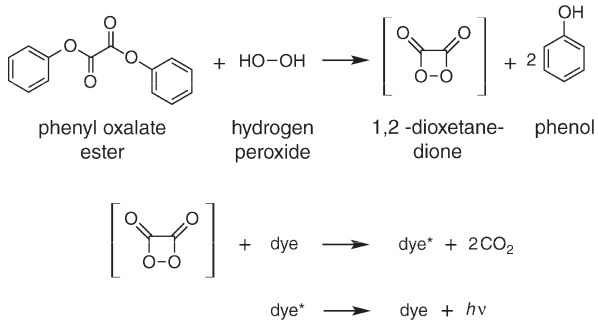
Many everyday consumer products, such as dietary supplements, functional foods, or even iced teas, attempt to attract the customers' attention by advertising the protective function of the antioxidant ingredients (e.g., ascorbic acid or flavonoids such as naringin). Antioxidants are defined as "organic compounds that are added to oxidizable organic materials to retard oxidation and, in general, to prolong the useful life of the substrates" *(1)*. Thus, an experiment examining antioxidants is appropriate vehicle to engage high school and undergraduate students in redox chemistry *(2, 3)*.

Modern food chemistry offers several established methods such as the TEAC- *(4)*, FRAP- *(5)*, or ORAC-assay *(6)* to quantify antioxidative capacity. However, these methods are expensive and use significant lab time because they photometrically measure the oxidative decrease of the concentration of a specific dye (FRAP, iron(III)−tris(pyridyl) triazin; ORAC, fluorescein; TEAC, 2,2′-azinobis(3-ethylbenzothiazoline-6-sulfonate)). This oxidative decrease of the dye concentration is influenced by the presence of antioxidative compounds, whose capacity is related to a reference called Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid), which is a water-soluble derivative of vitamin E. Similar attempts to measure the antioxidative capacity of compounds spectrophotometrically in undergraduate courses have been described in a recent issue of this *Journal (7)*.

This article describes a simple, reliable, and inexpensive procedure to qualitatively demonstrate the antioxidative capacity using the well discussed and impressive chemoluminescence reaction of lightsticks *(8−10)*. Lightsticks are available from most camping supply stores, sporting goods stores, and discount stores for under $1.

When a lightstick is snapped it shows a bright chemoluminescence. This luminescence results from the reaction of the hydrogen peroxide (from the glass vial in the lightstick) with the phenyl oxalate ester (in the plastic tube). The ester is oxidized, yielding two molecules of phenol and one molecule of 1,2-dioxetanedione, a high energy CO_2 dimer. The dimer spontaneously decomposes to carbon dioxide, releasing energy that excites the dye. The dye then relaxes by releasing a photon (Scheme 1). The wavelength of the photon, the color of the emitted light, depends on the structure of the dye *(11)*.

Scheme 1. The Unhindered Chemoluminescence Reactions of Lightsticks As Observed in the Blank Test



Experimental Procedure

The lightstick (15 mm × 150 mm; yellow lightsticks work best) is snapped and the contents are shaken well. The top of the lightstick is cut off with a metal cutter. The mixed content is filtered through a wire netting and apportioned into two test tubes. A small volume of pentane, 2 mL, is added to each test tube. To one test tube, 1 g of test substance (Table 1) is added and simultaneously, to the second test tube, 1 g of sodium chloride, which functions as the control substance, is added. Both test tubes are capped, shaken well, and placed back in the test tube holder. The difference of the light emission is compared after approximately 3 min.

Hazards

This experiment involves the use of irritating (the fluorescent dye used in the lightstick, for example, 1,8-dichlor-9,10-bis(phenylethynyl)anthracene in yellow lightsticks), harmful (pentane and sodium salicylate, serving as a catalyst in the lightstick), toxic (dibutyl phthalate, the lightsticks solvent), corrosive (hydrogen peroxide), oxidizing (hydrogen peroxide), and highly flammable (pentane) chemicals. While this experiment is performed, there should be no open flames in the laboratory. Students performing this experiment should wear gloves, safety glasses, and lab coats.

Discussion and Results

After the addition of the test substance, the light intensity of the batch shows an obvious decay (compare Table 1) compared to the blank test, which shows the normal luminescence of a lightstick (Figure 1). The observed decay of the light intensity after the addition of the assay can be traced back to its antioxidative capacity. By being oxidized themselves, antioxidative substances suppress the oxidation of the phenyl oxalate ester by hydrogen peroxide. As a consequence no 1,2-dioxetanedione is produced, the dye is not excited, and the glowing reaction does not occur or only occurs to a clearly diminished extent.

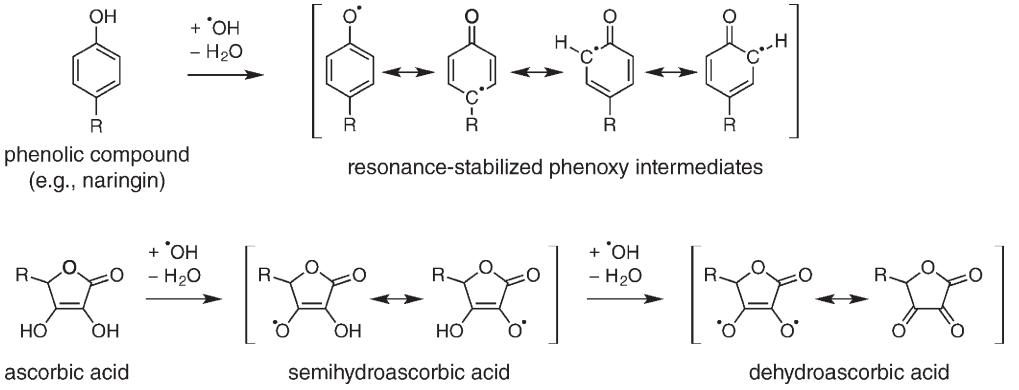
In most cases, the antioxidative capacity of a substance can be explained by its ability to form resonance-stabilized intermediates in the presence of radical formers. Because of their resonance-stabilization, these intermediates do not lead to a propagation reaction. By their ability to bind a second radical, many

10.1021/ed900043a Published on Web 01/25/2011

Table 1. Substances Tested with the Lightstick

Substance/1 g	Purchased at	Active Ingredients	Light Intensity (t = 3 min)
Black tea	grocery store	~150 mg of quercetin and catechin	low
Green tea	grocery store	~300 mg flavanols such as catechin	low
Grapefruit peel (dried and chopped)	grocery store	~50 mg naringin; ~3 mg vitamin C	high
Multivitamin pills (ground)	drug store	~100 mg vitamin C; ~15 mg vitamin E; ~1 mg vitamin A	high
Vitamin C (ascorbic acid)	Sigma Chemical Co.	1 g vitamin C	very high
Flavonoid (naringin)	Sigma Chemical Co.	1 g flavonoid	very high
Sodium chloride	grocery store	1 g of table salt	extremely high

Scheme 2. Resonance-Stabilized Intermediates of Ascorbic Acid and the Flavonoid Naringin



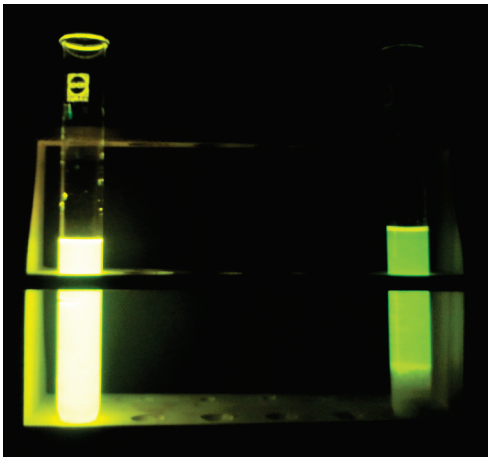


Figure 1. Two test tubes after the addition of a control or test substance: (left) sodium chloride control and (right) naringin.

antioxidants even cause the termination of the free radical reaction. This ability is exemplarily shown for ascorbic acid and naringin in Scheme 2. Phenolic compounds such as naringin can easily form four stabilized phenoxy intermediates, whereas ascorbic acid forms the resonance-stabilized semihydroascorbic acid in the first step and dehydroascorbic acid in the second step when radicals are present.

The value of the experiment is based on its simplicity. It is low cost, has a short preparation time, and can be performed in less than 5 min, either by the students or as a demonstration. The experiment has been successfully tested with 94 students in chemistry and biology in German secondary schools. In nearly all cases, it led to an obvious and impressive observation. The experiment is suitable for upper-level high school students who have studied inorganic redox reactions. The students are asked to apply their knowledge to organic topics, specifically the antioxidative capacity of substances such as ascorbic acid.

Acknowledgment

We would like to thank the "Fonds der Chemischen Industrie" (Mainzer Landstrasse 55, 60329 Frankfurt am Main, Germany) for the financial support of this work.

Literature Cited

1. *Ullmann's Encyclopedia of Industrial Chemistry*, 2nd ed.; Renner, H., Ed.; Wiley-VCH: New York, 2001.
2. Beaver, B. *J. Chem. Educ.* **1999**, *76*, 1108.
3. Donelly, T. H. *J. Chem. Educ.* **1996**, *73*, 158.
4. Rice-Evans, C.; Miller, N.; Paganga, G. *Free Radical Biol. Med.* **1996**, *20*, 933.
5. Benzie, I.; Strain, J. J. *Anal. Biochem.* **1996**, *239*, 70.
6. Glazer, A. *Anal. Biochem.* **1989**, *177*, 300.
7. Canelas, V.; da Costa, C. T. *J. Chem. Educ.* **2007**, *84*, 1502.
8. Kuntzleman, T. S.; Comfort, T. S.; Baldwin, B. W. *J. Chem. Educ.* **2009**, *86*, 64.
9. Roser, C. E; McClusky, C. L. *J. Chem. Educ.* **1999**, *76*, 1514.
10. Salter, C.; Range, K.; Salter, G. *J. Chem. Educ.* **1999**, *76*, 84.
11. Gill, S. *Aldrichimica Acta* **1983**, *16*, 59.

Supporting Information Available

Student spreadsheets; a list chemicals and equipment; hazards and safety instructions; instructor notes. This material is available via the Internet at http://pubs.acs.org.

