

# An Iodine Fluorescence Quenching Clock Reaction

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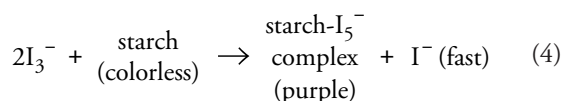
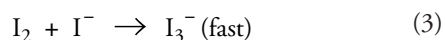
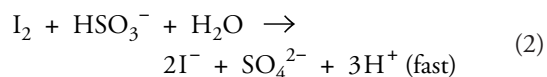
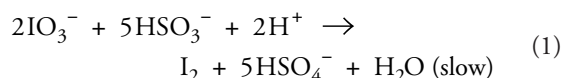
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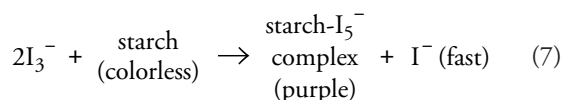
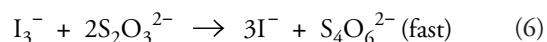
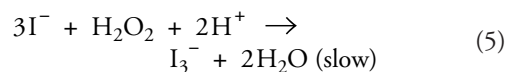
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Clock reactions are one of the most popular types of chemistry demonstrations, and instructions for conducting them are widely available in textbooks (1) and on the Internet (2). Not only do clock reactions provide crowd-pleasing, visually-dramatic color changes, but they provide an engaging illustration of redox phenomena, reaction kinetics, and the principles of chemical titration. Consequently, clock reactions can form the basis for classroom lab exercises suitable for a broad age range (3).

The largest class of clock reactions is based upon competing oxidation and reduction reactions of iodine and uses the formation of a complex between triiodide ( $I_3^-$ ) and starch to provide the final deep purple color (1). In the classic clock reaction originally described by Landolt (4), iodine species are simultaneously oxidized and reduced by bisulfite:



Shakhashiri described a popular variation of the Landolt reaction that uses iodide, hydrogen peroxide as the oxidizer, and thiosulfate as the reducing agent (5):

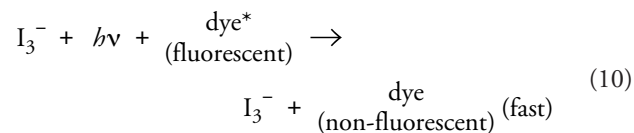
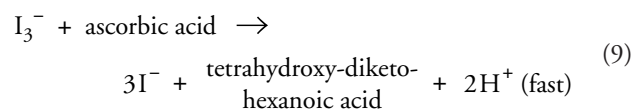
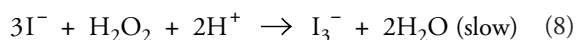


Many other variations of the iodine-based clock reaction have been reported, including one that is timed to symphonic music (6), one formulated to display school colors (7), and one that uses ascorbic acid from vitamin C tablets as the reducing agent (8).

A particularly dramatic type of clock reaction is the chemiluminescent clock reaction, in which two colorless solutions are mixed in a darkened room, followed after an interval by the sudden appearance of a blue luminescence (9). However, this reaction requires luminol, concentrated alkalis, and potassium tricyanocuprate, all of which are potentially toxic, require careful disposal, and are available only from chemical supply companies.

This luminescent clock reaction is based on easily obtained household consumer chemicals. In considering a feasible approach, I took note of the fact that not only is triiodide a much more effective fluorescence quencher than iodide (10), but also that it has a peak molar absorbance at 351 nm (11), which is in the middle of the long wavelength ultraviolet (UV-A) range. These properties suggested that substituting a suitable fluorescent dye for starch in an iodine clock reaction and irradiating the mixture with long wavelength UV, would result in a fluorescence emission that would be suddenly extinguished by triiodide after an interval determined by the concentration of reactants.

Hence I sought a fluorophore that (i) was readily available from commercial sources; (ii) had a peak excitation wavelength in the long wavelength UV range (315–400 nm); (iii) was compatible with the consumer product-based hydrogen peroxide–iodine–ascorbic acid clock reaction described by Wright (8); and (iv) ideally, in the spirit of emulating the classic luminol chemiluminescence reaction, exhibited a blue fluorescence emission (9). This reaction would proceed as follows:



I first considered fluorescein, a ubiquitous dye used in highlighter markers and antifreeze. Fluorescein has an excitation maximum at 490 nm, but it does fluoresce under long wavelength UV. However, fluorescein proved unsuitable because it undergoes a dramatic loss of fluorescence at acid pH (12). A product possessing all of the desired attributes is tonic water, which contains quinine, a strongly fluorescent compound that has an excitation maximum at 350 nm and fluorescence emission at 450 nm in dilute acid (13). However, experiments with several brands of regular and no-cal tonic water gave unsatisfactory fluorescence quenching kinetics, possibly owing to interference by other ingredients in the soda. Moreover, it was not possible to increase the concentration of quinine in the reaction mixture to achieve a brighter fluorescence.

I then recalled the fact that most commercial laundry detergents contain fluorescence whitening agents (also called optical brighteners). These highly fluorescent dyes bind tightly to fabrics, absorb long wavelength UV, and emit a strong blue fluorescence that neutralizes yellowness in laundered clothing (14). Two agents, FWA-1 {disodium 4,4'-bis[(4-anilino-6-morpholino-1,3,5-triazin-2-yl)amino]stilbene-2,2'-disulfonate} and FWA-5 {disodium 4,4'-bis-(2-sulfostyryl)biphenyl}, account for most of the optical brighteners used in detergents in the United States (15, 16).

Trial experiments revealed that when irradiated with long wavelength UV, aqueous solutions of a liquid laundry detergent emitted a blue-violet fluorescence at 432 nm, even at dilutions >1:2000, with a peak excitation wavelength of 350 nm.<sup>1</sup> Moreover, I found that acidification of detergent solutions caused a 50% increase in fluorescence intensity and an emission wavelength red shift to 457 nm, yielding a bright azure glow that was reminiscent of luminol chemiluminescence. This fluorescence was effectively quenched by even very small amounts of iodine. Further experimentation optimized the iodine fluorescent quenching clock reaction, as described below.

## Experimental Section

The following materials were obtained from local drugstores and supermarkets: white vinegar (5% acetic acid); 3% hydrogen peroxide USP; 2% iodine tincture USP; 500 mg vitamin C tablets (unflavored and uncoated); Arm & Hammer perfume and dye-free liquid laundry detergent.<sup>2</sup> Additional materials that are needed include kitchen measuring spoons; small containers such as shot glasses or clear plastic 4-ounce drinking cups; glass or plastic stirring rods; a hand-held long wavelength UV "black light" source; plastic graduated cylinders (optional); and volumetric pipets (optional).

### Preparation of a Vitamin C Stock Solution

A vitamin C stock solution is made by dissolving one 500-mg vitamin C tablet in 1 tablespoon of water in a small glass. The undissolved excipient solids are left to settle to the bottom of the glass, and the clear supernatant is decanted off into a clean glass.

### Procedure A

In this procedure chemicals are added sequentially to illustrate the individual steps and mechanisms of the reaction.

Measurements are made using standard kitchen measuring spoons. In a darkened room, the following are added to a small glass irradiated by a long wavelength UV source with gentle stirring after each addition:

- 1) 2 tablespoons of 3% hydrogen peroxide
- 2) 1/8 teaspoon of liquid laundry detergent

The fluorescence whitening agent in the laundry detergent will cause the solution to fluoresce with a blue-violet light.

- 3) 1 tablespoon of white vinegar

The fluorescence emission will noticeably brighten and change to a bright azure hue. pH titrations established that this fluorescence transition occurs between pH 4.8–4.6,<sup>1</sup> which is close to the predicted  $pK_a$  of the substituted 1,3,5-triazine rings present in FWA-1 (17). This suggests that protonation of the triazine rings at the pH of vinegar (2.5–3.0) causes an intramolecular charge transfer that alters its optical properties (18).

- 4) 1/4 teaspoon of 2% iodine tincture

The solution will immediately go dark as the  $I_3^-$  in the iodine tincture quenches the fluorescence by a combination of collisional energy transfer and an inner filter effect caused by the superimposition of the  $I_3^-$  UV absorption and FWA-1 excitation bands.

- 5) 1/4 teaspoon of vitamin C solution

The bright azure fluorescence will immediately reappear as the ascorbic acid reduces  $I_3^-$  to iodide, which not only is a much less efficient quencher,<sup>3</sup> but also has negligible UV-A absorption (19). Then, after about 10–20 seconds, when all the ascorbic acid has been consumed, iodide is rapidly re-oxidized to  $I_3^-$ , and the fluorescence will abruptly vanish. Adding more vitamin C to the darkened reaction will cause the fluorescence to reappear and go through another clock cycle. If the fluorescence does not fade rapidly enough, more iodine can be added. Conducting the reaction in daylight enables one to see the sudden appearance of the reddish-brown color characteristic of triiodide.

### Procedure B

This procedure produces a more dramatic demonstration. Two solutions are combined in the dark: a bright azure fluorescence appears, and then suddenly disappears. Solution #1 consists of 2 tablespoons of 3% hydrogen peroxide, 1/8 teaspoon of liquid detergent, and 1/4 teaspoon of iodine tincture. Solution #2 consists of 1 tablespoon of white vinegar and 1/4 teaspoon of vitamin C stock solution. The lights are dimmed, and the two solutions are simultaneously poured into an empty glass irradiated by a long wavelength UV "black light". The appearance-disappearance of fluorescence is most dramatic if the UV light has a built-in dark filter to minimize visible light emitted from the source. As above, additional clock cycles can be produced by addition of more vitamin C. For scaled-up volume reactions, the detergent should be present at a 1:100 dilution.

### Procedure C

In this theatrical demonstration, ascorbic acid crystals and volumetric measurements are used to achieve more precise control of the timing of the fluorescence quenching. Pairs of small plastic cups or shot glasses are set up in two parallel

rows. An ascorbic acid stock solution is made by dissolving 500 mg of ascorbic acid crystals (available at health food stores) in 15 mL of water. An adjustable volumetric pipet is then used to add 1.5 mL of ascorbic acid solution to each cup in the front row. Then an additional 0.1 mL is added to the second cup, 0.2 mL to the third cup, 0.3 mL to the fourth, and so on. A mixture of 30 mL of 3% H<sub>2</sub>O<sub>2</sub>, 15 mL of white vinegar, 1.5 mL of 2% iodine tincture, and 0.5 mL of liquid laundry detergent is added to each cup in the back row.

To perform the demonstration, the lights are dimmed, and the cups are illuminated with a long wavelength UV "black light". The peroxide-iodine-detergent mixtures are quickly poured into the cups containing the ascorbic acid, producing a line of glowing solutions. After about 30 seconds the fluorescence in each cup will "turn off" in sequence.

### Procedure D

Oscillating reactions are arguably the most dramatic of all chemical demonstrations, and of these, the Briggs–Rauscher reaction is perhaps the best known (20). The oscillating color changes in this reaction (from clear to amber to deep blue) are caused by the redox cycling of iodide → iodine → triiodide in the presence of soluble starch as an I<sub>3</sub><sup>-</sup> indicator. The similarity of this chemistry to the H<sub>2</sub>O<sub>2</sub>–iodine clock reaction immediately suggested that substitution of liquid laundry detergent for starch in the Briggs–Rauscher reaction would yield an oscillating fluorescence due to cyclic I<sub>3</sub><sup>-</sup> fluorophore quenching.

To evaluate this possibility, I used a commercially available Briggs–Rauscher reaction kit<sup>4</sup> that not only uses small quantities of chemicals, but also uses 3% hydrogen peroxide and sulfamic acid instead of concentrated H<sub>2</sub>O<sub>2</sub> (30%) and sulfuric acid (21). I followed the kit instructions with no modifications except that I added several drops of liquid detergent instead of the provided soluble starch solution and viewed the reaction under long wavelength UV. As anticipated, the modified reaction fluoresced, abruptly went dark, and then slowly glowed again with an oscillation period of ~10 seconds.

### Hazards

Hydrogen peroxide, H<sub>2</sub>O<sub>2</sub>, is a powerful oxidizer, even at a 3% concentration (22). It can cause skin and eye irritation and nausea and abdominal pain if ingested. Under certain conditions it can violently decompose, releasing oxygen, so it should be kept away from open flames. Iodine tincture is a poisonous oxidizing agent that can stain skin and clothing (23). It is irritating to open cuts and can cause severe inflammation if splashed in the eyes. It is toxic if ingested and can cause nausea, vomiting, and abdominal pain. Inhalation of iodine vapors can cause coughing and difficulty breathing. As the Briggs–Rauscher reaction evolves iodine vapor, procedure D should be conducted in a well-ventilated room. Liquid laundry detergent is irritating to mucous membranes, particularly the eyes. UV light can cause corneal irritation and retinal damage. Eyes should be shielded at all times from direct exposure to any UV light source, optimally by UV safety glasses. Never look directly at a UV light source. At the conclusion of the demonstration, all iodine containing solutions should be treated with an excess of vitamin C until they are fully decolorized before disposal.

### Comments

The examination of the photophysical principles underlying the iodine fluorescence quenching clock reaction can provide opportunities for classroom study beyond the published exercises designed to explore the kinetics of iodine redox reactions (3). Students may be surprised to learn that the reason that their clothes fluoresce under UV light is that they have been washed in detergents that contain fluorescent dyes. Indeed, the pervasive use of fluorescent whitening agents and the mass disposal of these chemicals in laundry waste water is an environmental concern (15, 16).

Many students will be familiar with pH indicators that change color in acidic or basic conditions; however, they are likely less to be aware of the fact that pH can alter the properties of fluorescent molecules. Such fluorescent pH indicators are used in biomedical research to measure the pH inside of living cells (24). Students can determine the pH at which the fluorescence emission of liquid detergent changes color by adding detergent to a series of pH buffered solutions under UV light<sup>5</sup> or by titrating detergent solutions under UV light with dilute vinegar while monitoring the pH with narrow range pH paper.

Students can also explore the qualitative stoichiometry of the fluorescence quenching by observing the progressive decrease in fluorescence intensity of 1:100 dilution of detergent in 30 mL of vinegar as increasing quantities of iodine tincture are added with a disposable plastic dropper under UV light. The fluorescence will be totally quenched after only ~5 drops of iodine tincture have been added. If the experiment is repeated using iodine tincture that has been reduced by addition of ascorbic acid, even after addition of 10 mL of reduced iodide, the fluorescence will still be visible. The potent ability of triiodide to quench fluorescent dyes forms the basis of analytical techniques to measure extremely small quantities of iodine in foods and water (10).

### Notes

1. The wavelengths of maximum excitation and emission of the fluorophore in liquid laundry detergent were determined using an SLM 8000 spectrofluorometer. The effect of pH on the maximum emission wavelength and peak emission intensity was examined with 1:100 dilutions of detergent in 10 mM acetate buffers over a pH range of 3.5–6.0.

2. Many other detergents contain fluorescent whitening agents (14). As long as the detergent is clear (i.e., does not contain other dyes or additives that might cloud or obscure the fluorescence emission) it may be substituted in the reaction mixture.

3. The fluorescence quenching efficiency of iodide and triiodide was determined by monitoring the peak fluorescence of a 1:50 dilution of liquid detergent in 10 mM acetate buffer, pH 3.5, as aliquots of 5 M KI or 0.5 M triiodide solution were added. Stern–Volmer fluorescence quenching constants,  $K_Q$ , were calculated from the slope of plots of  $F_0/F$  versus  $[I]$ , where  $F_0$  is the initial fluorescence intensity and  $F$  is the fluorescence intensity at a given iodide or triiodide concentration,  $[I]$  (25).  $K_Q$  for iodide was 2.39;  $K_Q$  for triiodide was 1469. Thus, triiodide was 615-fold more potent than iodide in quenching the fluorescence of liquid detergent.

4. Educational Innovations, catalog no. CK-475, Fascinating Oscillating Reaction Kit. <http://www.teachersource.com/catalog> (accessed Feb 2007).

5. Small UV light-emitting diodes with maximum emission between 395 to 405 nm can work well for students in a classroom laboratory setting and are widely available on the Internet. A reliable source is <http://www.stevespanglerscience.com/product/1433> (accessed Feb 2007).

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