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Communication

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# General Chemistry Laboratory Experiment To Demonstrate Organic Synthesis, Fluorescence, and Chemiluminescence through Production of a Biphasic Glow Stick

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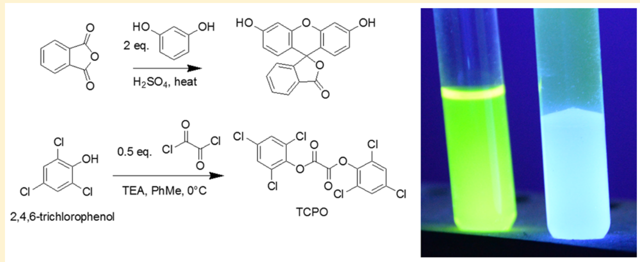
Supporting Information

**ABSTRACT:** A laboratory experiment is described for beginning, nonmajor chemistry students, which allows students to examine the phenomena of fluorescence and chemiluminescence, as well as gain experience in basic organic synthesis. Students synthesize fluorescein and bis(2,4,6-trichlorophenyl) oxalate (TCPO) to explore fluorescence and chemiluminescence by assembling a biphasic glow stick. The experiment gives students the opportunity to understand the chemistry behind common "real-life" experiences of glow stick illumination and fluorescent lighting or inks and to synthesize compounds that exhibit these phenomena. Students' survey data between 2013 and 2016 show that the experiment was enjoyable to undergraduate nonscience majors (4.3 of 5 cumulative, 4.8 in 2016) and relevant to their lives (4.0 of 5 cumulative, 4.4 in 2016).



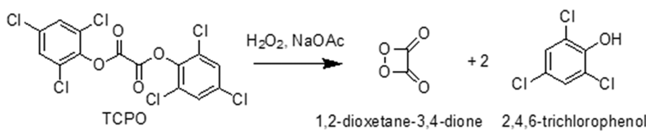

**KEYWORDS:** *Organic Chemistry, First-Year Undergraduate/General, Hands-On Learning/Manipulatives, Applications of Chemistry, Synthesis, Laboratory Instruction*

## ■ INTRODUCTION

Introducing students to organic reactions in the laboratory is useful to correlate the theoretical study of chemistry to its practical applications, to instill proper laboratory techniques, to provide the opportunity for hands-on experience, as well as to investigate interesting chemical phenomena by relating laboratory work to "real-life" experiences. This laboratory experiment focuses on the synthesis of compounds which produce fluorescence and chemiluminescence through the production of a biphasic fluorescent and chemiluminescent glow stick. Although there are several laboratories and demonstrations which take advantage of fluorescence and chemiluminescence separately[1] as well as the synthesis of dyes and fluorophores,[1j,2] this experiment combines multiple synthetic organic reactions to fabricate a biphasic glow stick, in addition to in-depth exploration of fluorescence and chemiluminescence. The synthesis of bis(2,4,6-trichlorophenyl) oxalate (TCPO; used for energy generation in glow sticks to produce chemiluminescence) in a teaching laboratory setting is novel and teaches basic laboratory techniques used in organic synthesis.

Students may be familiar with the process of fluorescence with fluorescent lighting, fluorescent ink (e.g., on t-shirts or in books), and/or black light illumination. Chemiluminescence is similar to fluorescence, but excitation occurs via energy produced from a chemical reaction. Many students will have experienced chemiluminescence from glow sticks, although they may be unaware of the connection. When used in glow sticks, TCPO reacts with hydrogen peroxide under basic conditions, yielding 1,2-dioxetane-3,4-dione (Scheme 1). This

**Scheme 1. Decomposition of TCPO into 2,4,6-Trichlorophenol and 1,2-Dioxetane-3,4-dione**




compound decomposes in solution, producing carbon dioxide and exciting the $\pi$ system of a fluorophore (e.g., 9,10-diphenylanthracene) to a higher energy level.[3] Like fluorescence, the excited electron relaxes to its ground state, and a photon is released, causing chemiluminescence (Scheme 2). Glow sticks of different colors can be made using dyes which emit different wavelengths of light.[4] This experiment combines the phenomena of fluorescence and chemiluminescence. The glow stick features a fluorescent aqueous upper layer which is excited by an organic chemiluminescent lower layer. Instructor notes and a detailed laboratory procedure are available as Supporting Information.

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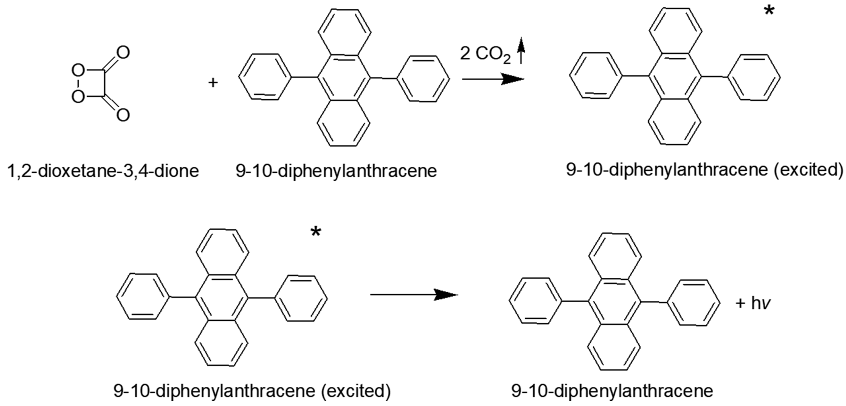
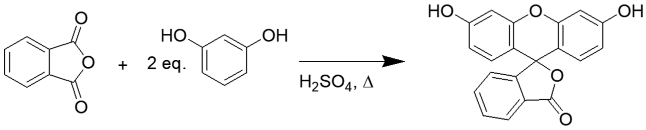
**Scheme 2. Reaction of 1,2-Dioxetane-3,4-dione and 9,10-Diphenylanthracene To Produce the Energy Required for Chemiluminescence**




## Synthesis of Fluorescein

Students first synthesize fluorescein, as shown in Scheme 3. This simple and expedient synthesis is an acid-catalyzed alkylation of phthalic anhydride with resorcinol where the two solids are added to a test tube with a small amount of sulfuric acid and heated directly on a hot plate.[5] The solids melt and react to produce fluorescein which absorbs blue light ($\lambda_{max}$ = 494 nm) and emits green light ($\lambda_{max}$ = 521 nm).[6]

**Scheme 3. Synthesis of Fluorescein from Phthalic Anhydride and Resorcinol**




Fluorescein undergoes tautomerization where the predominant tautomer varies with respect to pH. The structural differences between the tautomers affect their fluorescence because, in order to possess fluorescence, a molecule must possess a highly conjugated bond system, i.e., a system of alternating single and double bonds. As shown in Figure 1 (left), the formation of a lactone under acidic to neutral conditions interrupts the molecule's conjugated system, preventing fluorescence.[7] Therefore, to observe fluorescent behavior, fluorescein must be dissolved in basic solution to ensure that it is deprotonated (Figure 1, right). Once the fluorescein is synthesized, the students make a basic solution and evaluate its fluorescence behavior. The synthesized fluorescein is shown (Figure 2, left test tube in each panel) under room light (Figure 2A), in the dark (Figure 2B), and under UV/black light (Figure 2C). Some small amount of fluorescence (yellowish "glow"), especially near the top of the solution, is present under room lighting (Figure 2A) and from the chemiluminescence in the dark (Figure 2B). Fluorescence is clearly evident under UV/black light irradiation from above (Figure 2C).

## Synthesis of TCPO

The second reaction performed in this experiment (Scheme 4) is the synthesis of TCPO via an esterification reaction between 2,4,6-trichlorophenol and oxalyl chloride in toluene.[8] TCPO is used to provide the energy required for the chemiluminescence observed in glow sticks (Schemes 1 and 2). This reaction is carried out in an Erlenmeyer flask sealed with a septum to minimize exposure to air and moisture. In addition to providing students with experience in organic synthesis, this reaction demonstrates the importance of careful control of reaction conditions, since the oxalyl chloride may easily be hydrolyzed into oxalic acid rather than produce the desired product. Moreover, the oxalyl chloride must be added dropwise, or the energy produced by the reaction in Scheme 4 will cause a buildup of pressure, potentially breaching the septum used to seal the reaction vessel.

## Glow Stick Construction

The final step in the experiment is to assemble a laboratory-based, biphasic glow stick in a test tube. The synthesized TCPO is added to a solution of 9,10-diphenylanthracene (the chemiluminescent dye) and sodium acetate dissolved in diethyl phthalate. Hydrogen peroxide (30% w/v) is added to this solution, and an aqueous solution of fluorescein is then added to the top of this organic layer; this forms a biphasic mixture with a lower chemiluminescent organic layer and an upper fluorescent aqueous layer (Figure 2, right test tube in each panel). The chemiluminescent lower layer produces blue light, which is absorbed by the fluorescent upper layer and re-emitted as green light.

## ■ HAZARDS

Students should exercise appropriate precautions when handling the chemicals in this lab. Specifically, students should

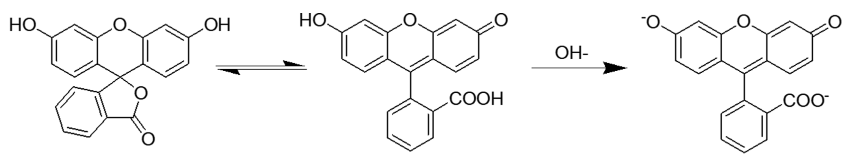



**Figure 1.** Tautomeric forms of fluorescein in acidic (left) and neutral (center) conditions. The anionic form is present in basic (right) conditions. The lactone present in acidic conditions prevents fluorescence by interrupting the molecule's conjugation.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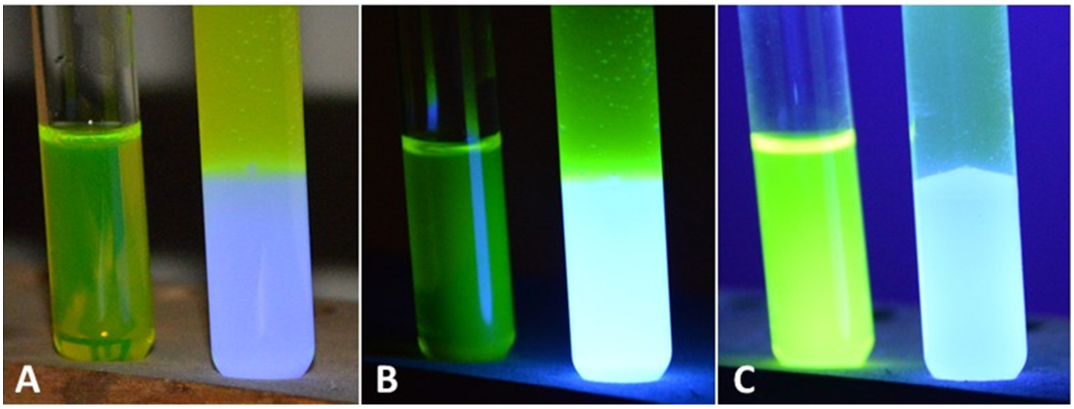
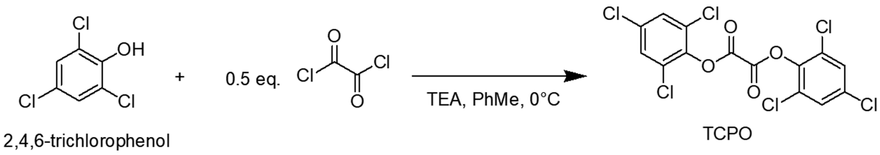



**Figure 2.** Solutions under room light (A), in darkness (B), and under UV/black light illumination from above (C). In each photo, the fluorescein is on the left, and the glow stick is on the right.

**Scheme 4. Synthesis of TCPO from 2,4,6-Trichlorophenol and Oxalyl Chloride**




wear goggles, gloves, and appropriate lab attire for the entire laboratory period. Concentrated sulfuric acid is corrosive and oxidizing, can produce hazardous fumes, cause chemical burns, and should be handled in a well-ventilated fume hood. Concentrated hydrogen peroxide can cause chemical burns, and contact with skin should be avoided. Toluene and triethylamine are highly flammable and should be kept away from ignition sources. Oxalyl chloride is highly water sensitive, will react vigorously with water, and should be handled accordingly. The addition of the oxalyl chloride during the synthesis of TCPO must be performed carefully and slowly to avoid the exothermic reaction producing thermal runaway and causing a pressure buildup from boiling solvent. Oxalyl chloride is an irritant; the use of a fume hood is recommended. TCPO and fluorescein are both toxic, and contact with skin and eyes should be avoided. Care should be taken when handling the organic solution containing the fluorescent dye as well. Diethyl phthalate can be flammable at high temperatures, and 9,10-diphenylanthracene can be an irritant when in contact with skin or eyes.

## ■ SUMMARY

Students produce a biphasic glow stick in this laboratory experiment, which demonstrates the concepts of fluorescence, chemiluminescence, and synthetic organic chemistry. The laboratory also serves to demonstrate a practical use of organic synthesis in the production of dyes and other chemicals. The pedagogic goals for the laboratory experiment were for students to learn to (1) perform synthetic organic methods to synthesize most of the component reagents of the glow stick, (2) construct a biphasic glow stick from component reagents, and (3) describe the chemical and physical properties of chemiluminescence and fluorescence. All the students performing this lab from 2013 to 2017 ($N$ = 299) synthesized fluorescein. While the TCPO synthesis was more problematic (i.e., for reasons described above and in the Supporting Information), approximately 95% of the students were able to synthesize this compound via the laboratory procedure. All students constructed the biphasic glow stick and investigated its properties, with TCPO provided to those students who failed to synthesize it. The realization of goal 3 was assessed by asking the students to describe the similarities and differences of chemiluminescence and fluorescence in the prelab and using two application/analysis questions postlab. Students were asked the following: (1) Why was the fluorescence intensity of the fluorescein brighter at the interface between the two layers of the biphasic glow stick than in the rest of the fluorescein layer? (2) From where does the energy seen as chemiluminescence come? While students struggled on the prelab question, they were better equipped to answer the postlab questions, increasing their scores on the postlab questions significantly. Following the lab period, students were given a survey of their impressions on the experiment and were allowed to provide comments. Data were collected between 2013 and 2017 from 299 students who completed the laboratory. In general, the students commented that the lab was an engaging and enjoyable experience with 60% of the students surveyed specifically mentioning that the lab was fun, interesting, and/or enjoyable when asked an open-ended question about what aspects should remain in the laboratory experiment in the future. Students also rated the lab a cumulative (2013–2016) 4.3 out of 5, with a rating of 4.8 in 2016, when asked if they agree with the statement that the lab was enjoyable on a Likert scale (5, strongly agree; 1, strongly disagree). Students also appreciated the relevance to their everyday lives, rating the lab a 4.0 of 5 (cumulative; 4.4 in 2016) when asked if they agree with the statement that the lab was helpful to understand something they have wondered about at some point in their lives.

## ■ ASSOCIATED CONTENT

### Supporting Information

The Supporting Information is available on the ACS Publications website at DOI: 10.1021/acs.jchemed.7b00194.

Laboratory procedure (PDF, DOCX)
Instructor notes (PDF, DOCX)



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### Notes

The authors declare no competing financial interest.


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