Extraction of dyes contained in glow sticks using liquid CO\textsubscript{2}

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**ABSTRACT**

Separation of glow stick dyes adhered to a cotton swab using liquid CO\textsubscript{2} provides an engaging demonstration of several chemical concepts including polarity, kinetics, chemiluminescence, and the importance of CO\textsubscript{2} as a green solvent. The simple protocol allows access to a broad range of students. Differential polarities of the glow stick dyes allow certain dyes to be preferentially dissolved in liquid CO\textsubscript{2}, leaving other dyes adhered onto cotton. Both TCPO (bis(2,4,6-trichlorophenyl)oxalate) and H\textsubscript{2}O\textsubscript{2}, which together provide the necessary reaction for chemiluminescence, are present in both the extracted liquid and cotton upon dissipation of CO\textsubscript{2}. Thus, it is possible to compare the emission spectrum of the extracted fluid to that of the original glow stick and the residue left on the cotton swab. Emission peaks resulting from the presence of polar dyes in the original glow stick and on the cotton are routinely observed to be missing in the extracts.

**Introduction**

Both liquid CO\textsubscript{2} and supercritical CO\textsubscript{2} can be used as a substitute for volatile organic solvents in extraction and chromatographic methods (1–7). CO\textsubscript{2} can be obtained from and returned to the air in a cyclic fashion, it is neither toxic nor flammable, and its use as a solvent avoids the production of large quantities of waste solvent (7). In fact, in a survey of academic researchers, CO\textsubscript{2} was identified as the solvent mostly likely to reduce environmental damage (8). Thus, the use of CO\textsubscript{2} as a solvent in separation science can provide avenues to introduce students to the importance of green chemistry and sustainability. Indeed, several experiments that involve the use of liquid CO\textsubscript{2} during extractions and chromatographic separations have appeared in the chemical education literature. For example, the use of liquid CO\textsubscript{2} to extract various organic compounds from orange peels (1,2), fennel seeds (3), cloves (4), and essential oils (5) has been previously reported.

Chemiluminescence is another topic that has been extensively covered in the chemistry curriculum (9–14). In fact, chemiluminescent reactions have even been referred to as the most “exocharmic” reactions known, based on the high interest they generate in students and other observers (9). Chemiluminescent reactions have been used to introduce students to topics as varied as fluorescence, kinetics, thermodynamics, chromatography, catalysis, organic synthesis, and principles of...
green chemistry (9–14). Many of these experiments have focused on the chemistry of glow sticks, which provide very easily obtained sources of chemiluminescent reactions. The use of such simple materials in experiments is attractive for many chemical educators who work at institutions that do not have large budgets or access to expensive equipment. In a previous publication, it was demonstrated how materials contained in an activated glow stick could be separated using column chromatography (9). In some cases it was possible for students to observe distinct, actively glowing bands on the column as the separation took place. This experiment was light-heartedly termed “glowmatography” on account of its combination of glow sticks and chromatography.

In this letter, we build upon the body of previously reported work to describe how liquid CO2 can be used to separate various dyes contained in glow sticks using materials as simple as dry ice, plastic centrifuge tubes (1), glow sticks, and cotton swabs. The development of laboratory exercises that use liquid CO2 rather than supercritical CO2 is important, because many chemical educators and their students do not have the resources or infrastructure necessary to carry out experiments using supercritical CO2. However, extractions involving liquid CO2 are quite easy to perform (1–5). Thus, laboratory experiments that incorporate liquid CO2 as a solvent allow a wider audience of students to learn about the potential benefits of CO2 as a solvent. Indeed, the experiments reported in this letter introduce students to the same chemical principles taught in the original glowmatography experiment, but without the use of hexane solvent and silica gel stationary phase. In addition, over ten times less glow stick material is required when using this new extraction method. Thus, this new method described represents a substantial move toward a more environmentally friendly study of separation of dyes contained in glow sticks.

Background

The light emitted from a glow stick is powered by the base catalysed reaction between bis(2,4,6-trichlorophenyl) oxalate (TCPO) and hydrogen peroxide (10) Figure 1. In the first step of the reaction mechanism, TCPO (11) reacts with hydrogen peroxide to form a reactive dioxetanedione intermediate, C2O4. This intermediate collides with a fluorescent dye molecule contained in the glow stick, and upon doing so promotes an electron in the dye to an excited state (dye*). As the electron relaxes back to the ground state, a photon of light is concomitantly emitted. Because the wavelength of the photon emitted depends upon the chemical structure of the dye, various colors of light emission are obtained by placing different dyes or mixtures of dyes within glow sticks (9). For example, various rhodamine dyes emit red or orange light, while bis(phenylethynyl)anthracene (BPEA) dyes emit yellow, green, or blue light. In addition to these differences in colors of light emitted, rhodamine dyes tend to be more polar than BPEA dyes (9). The experiments reported herein allow students to use liquid CO2, a non-polar solvent, to extract actively glowing non-polar dyes from an activated glow stick mixture. Direct visualization of the extraction process is possible because it is easy to recognize that the extracted material glows a different color than the original mixture.

Materials and methods

A glow stick (both Supreme Glow and Super Glow brands work, available at https://www.partycity.com/) is activated by breaking the inner glass ampoule. The glow stick is cut open using a PVC pipe cutter. A cotton swab with a plastic handle is cut in half, and the cotton end is dipped into the activated glow stick mixture. Excess liquid is dabbed from the cotton. The swab is placed cotton side up into a Corning 15 mL
polypropylene centrifuge tube (Fisher Scientific, manufacturing number 430052). Dry ice is powdered with a hammer, and then added to the centrifuge tube until it is roughly ¾ full. The cap is sealed tight and the assembly is immediately placed into hot water (60–70°C) contained in a 355 mL plastic soda bottle with its top removed. As the dry ice sublimes, pressures sufficient to liquefy CO₂ build inside the centrifuge tube, melting usually occurs within 30 s. Even so, pressure does vent from the tube as evidenced from considerable hissing noise observed as CO₂ gas escapes. On occasion, the dry ice does not liquefy. If not, it is helpful to tighten the seal of the cap on the centrifuge tube. Non-polar dyes contained in the glow stick mixture are preferentially dissolved into the liquid CO₂ that rinses through the cotton, and these dyes collect in the bottom of the centrifuge tube (Figure 2). If available, an emission spectrometer is used to collect the emission spectra of the glowing extract and the glowing residue remaining on the cotton. In addition, the emission spectrum of a second cotton swab that has been dipped into the activated glow stick mixture – but that has not gone through the CO₂ extraction process – is also recorded. All processes are carried out by students except the collection of emission spectra. Emission spectra were carried out in the dark using an Ocean Optics USB4000 spectrometer with an optical resolution of 1.0 nm FWHM; integration time was set at 5 s. Light emitted from extracts and material adhered to cotton swabs were collected via a fiber optic cable attached to the spectrometer. To collect spectra of material on cotton swabs, the end of the fiber optic was held ∼3 mm above the surface of the cotton swab. To collect spectra of residues in a centrifuge tube, the fiber optic was inserted into the centrifuge tube and held ∼3 mm above the surface of the extract (after removal of the cotton swab).

Results

Fluid from an activated, orange light stick is applied to a cotton swab and placed in a centrifuge tube as described in the Materials and Methods. This is an elegant manner of providing polar material that remains suspended above the bottom of the centrifuge tube. The stalk of the cotton swab is made of polypropylene, analysed by FTIR, and therefore neither of the dyes absorb onto the stalk, making analysis of both extract at the bottom of the centrifuge tube and the residue remaining on the cotton tip quite easy and convenient. The centrifuge tube is filled with powdered dry ice and sealed. When the liquid CO₂ forms, it drips through the cotton swab. The glowing stops due to the decrease in temperature, and the liquid CO₂ takes on a faint orange-yellow color from dyes that dissolve in the liquid CO₂ (Figure 2, left). Dyes extracted from the cotton collect on the bottom of the centrifuge tube as the CO₂ escapes. After all the CO₂ escapes, the cap is removed and the contents of the centrifuge tube are allowed to warm up. Upon reaching about room temperature, both the residue on the cotton and the extract begin to glow again (Figure 2, right). The light emitted from the cotton swab is a noticeably different color than the light emitted from the extract (Figure 2, right), due to differential solubility of dyes in the liquid CO₂. While the emission spectrum of the glowing residue adhered to the cotton (Figure 3, thin line) is somewhat dimmer than fresh glowing glow stick mixture adsorbed onto a cotton swab (Figure 3, bold line), the spectra are otherwise quite similar. Both spectra show peaks at about 550, 620, and 675 nm. On the other hand, the peaks at 620 and 675 nm are clearly diminished in the emission spectrum of the extracted material on the bottom of the centrifuge tube (Figure 3, dotted line). These results indicate that liquid CO₂ tends to extract yellow-green emitting dye (s), but not red emitting dye(s) from the cotton. Given the non-polar nature of liquid CO₂ and the polar nature of the cellulose in the cotton, it is likely that the red-orange emitting dye(s) are more polar than the yellow-green emitting dye(s). When the experiment is repeated using a pink light stick, the emission spectra of fresh glow stick mixture (Figure 4, bold line) and residue adhered to
the cotton (Figure 4, thin line) both show peaks at 445, 475, 620, and 675 nm. On the other hand, while the peaks at 445 and 475 nm appear in the emission spectrum of the extract, the peaks at 620 and 675 nm are almost absent (Figure 4, dotted line). These results suggest that liquid CO₂ extracts a substantial amount of non-polar, blue emitting dye(s) from the pink glow stick fluid, leaving red emitting dye(s) adsorbed on the cotton. The spectra are consistent with the more pronounced blue color observed in the extract than on the residue adhered to the cotton (Figure 5). This extraction method can be successfully applied using glow sticks of other colors, but orange and pink glow sticks tend to provide the greatest contrast in color between the extract and residue adhered to the cotton. Results from experiments using glow sticks of other colors, as well as a student laboratory sheet, can be found in the supporting information.

**Discussion**

Using liquid CO₂ to extract dyes from activated glow stick mixtures provides a simple, colorful, and motivating way to introduce students to the importance of sustainable solvents. The process is easy to carry out, allowing students at a wide variety of institutions to participate in these experiments. Nevertheless, the process allows for the discussion of several chemical topics including chemiluminescence, molecular polarity, solubility, kinetic mechanisms, emission spectroscopy, and intermolecular forces.

During the extraction process, it is interesting to note that the glow stick material adsorbed on the cotton loses its glow. This is not surprising, given that it is well known that the kinetics of the chemical reaction in glow sticks substantially decreases with temperature \(^{14,15}\). However, it is unexpected that both the residue on the cotton swab and the extract begin glowing again after being warmed back to room temperature following the extraction process (Figure 2, right and Figure 5). In order for chemiluminescence to occur, TCPO, H₂O₂, and dye(s) must be present (Figure 1). Therefore, both the cotton residue and extract contain TCPO and H₂O₂. The presence of TCPO in the extract is likely due to the fact that both TCPO and liquid CO₂ are non-polar. The
presence of H$_2$O$_2$ on the cotton is probably due to hydrogen bonding and other dipole–dipole interactions between the peroxide and cotton cellulose. That some H$_2$O$_2$ ends up in the extract implies that H$_2$O$_2$ dissolves in liquid CO$_2$. This may occur to some extent if O-H groups on the peroxide act as hydrogen bond donors, while the oxygen atoms on CO$_2$ act as hydrogen bond acceptors. Similarly, TCPO adhered to the residue could occur by O-H groups on cellulose and oxygen atoms on TCPO acting as hydrogen bond donors and acceptors, respectively. In any event, it is fortuitous that these compounds are present in both the cotton residue and extract, allowing both to recover easily visible chemiluminescence upon warming.

In conclusion, the experiments reported here provide an enlightening and colorful way for students to gain experience using CO$_2$ as an environmentally friendly solvent. Dyes in glow stick mixtures can be extracted somewhat selectively using simple and inexpensive materials. The protocol is straightforward enough that it can be carried out by non-science majors. On the other hand, interpreting the results of these experiments requires a knowledge of a rich assortment of physicochemical topics. As such, we have used this experiment in settings that range from outreach events to undergraduate research projects.

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Disclosure statement

No potential conflict of interest was reported by the authors.

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