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Laboratory Experiment

Glowing-in-the-Screen: Teaching Fluorescence with a Homemade Accessible Setup

Micaela Hamer, Agustina Maria Beraldi, Sergio G. J. Gomez, Facundo Ortega, Diego Onna,* and Mariana Hamer*

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ABSTRACT: Teaching fluorescence aims to deeply communicate light−matter interactions. The main fluorescence concepts are covered in undergraduate courses and are difficult to comprehend as the proper lighting conditions for seeing it by the naked eye are rarely found in daily life. Here, we present a lab activity to explore fluorescence using homemade equipment in order to complement the teaching of the basics of fluorescence and perform typical experiments. This device consists of a cardboard box as fluorometer with a flashlight as an excitation source and a smartphone camera with an application for color analysis as a detector. The presented assays can be used in diverse teaching environments to reinforce the universal concepts of fluorescence, Stokes shifts, quenching, and the inner filter effect. The assays are based on common products (such as green leaves, turmeric, tonic water, and salt), so access to the materials and waste generation are not a problem. The results, robustness, and reliability of this setup were discussed after evaluating the comments of teachers and students who perform the lab experiences. Furthermore, the performance of the students was assessed using a survey, which demonstrates this is an excellent approach to increase their interest and motivation for learning.

KEYWORDS: *High School/Introductory Chemistry, First-Year Undergraduate/General, Hands-On Learning/Manipulatives, Dyes/Pigments, Fluorescence Spectroscopy, Green Chemistry, Laboratory Equipment/Apparatus*

INTRODUCTION

The interaction of light with matter is an essential topic covered in undergraduate curricula as the importance of fluorometric methods is growing constantly. Most students usually have a clear understanding of the absorption phenomenon because it can be encountered in daily life. However, the fluorescence concept is less familiar and is difficult to comprehend as proper lighting conditions for observing it by the naked eye are rarer, leading to the necessity of laboratory experiments that reinforce and exemplify the explained concepts, even basic ones related to fluorometry.

Usually, hands-on activities for fluorescence teaching are limited due to the high costs of instrumentation, supplies, and reagents needed.[1] Moreover, modern instruments have become also "black boxes", which does not easy learning the concepts of spectroscopy.[2] Assays and experiments involving self-made equipment and newly accessible technology give an opportunity for students to grab complex topics and stimulate their curiosity.[3]

Following this line, many approaches in fluorescence portable devices have been described using light-emitting diodes (LED), optical filters, digital cameras, and smartphones, among other items, for teaching or research goals.[4−10] An interesting approach is an "open-box" fluorescence quenching method using an iPad screen as the excitation source and a digital camera as the detector,[2] while other authors have introduced a setup prepared with a shoebox and a flashlight as an excitation source.[6] Another report describes a homemade "fluorescence microscope" built up with a light-emitting diode (LED) and optical filters.[11] However, these approaches still have the inconvenience of using uncommon or unaffordable materials that do require special waste management[12−14] which especially is a concern for home lab experiences. Furthermore, the huge advance in smartphone technology has made the acquisition of high-quality scientific images accessible, together with the availability to analyze and manipulate them through free applications. This opens the possibility for developing innovative and comprehensive laboratory experiences.

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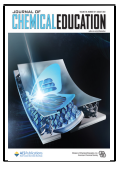

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Considering the importance of first-hand experiences in learning fluorescence, we developed a homemade fluorometer and hands-on experimental setup for practical demonstrations and lab assignments. Using this device contributes to comprehension of complex fluorescence concepts such as excitation and emission processes, Stokes shift, quenching, and inner filter effect (IFE) employing common commercial products.[15]

We also addressed two important points for a lab activity: (i) a simple and easy design of the setup and (ii) the use of accessible nontoxic nor polluting reagents.

Summing up, the learning objectives of this remote lab experience follow.

1. Recognize the fluorometers' arrangement:
   - Assemble their own fluorometer.
   - Identify radiation source, detector, sample holder, and light paths.
2. Give examples of the main aspects of fluorescence phenomena:
   - Determine the fluorescence of different compounds.
   - Interpret the effect of the physicochemical environment on the fluorescence intensity (solvent, pH).
   - Discuss the Stokes shift.
   - Distinguish quenching effect and apply the steady-state Stern−Volmer equation to calculate the Stern−Volmer quenching constant.
   - Demonstrate the IFE.

THEORY

In general terms, a fluorescent molecule absorbs light at a certain wavelength and then, after a finite time (usually nanoseconds), emits light at a longer wavelength.[16,17] This process involves the absorption of a photon by a molecule, leading to an electron transition from its ground electronic state to an excited energy level (eq 1).[15] Immediately, the molecule will relax back down to its ground-state emitting a new photon (eq 2), which has less energy than the absorbed photon because of a vibrational relaxation occurring in the electronic excited-state, or there will be heat emission (eq 2). This energy loss causes the emitted light to be at a longer wavelength than the absorbed one, an effect known as the Stokes shift. This difference depends on the fluorophore's chemical structure and its solvation environment. A simplified Jablonski diagram is usually used to explain the fluorescence process (Figure S1).[15]

Quenching is a process through fluorescence intensity and lifetime decrease. The most common type of quenching is described as a bimolecular process (eq 3), and it follows the kinetic scheme below:[18]

$$\mathrm{F} + h\upsilon_1 \rightarrow {}^*\mathrm{F} \tag{1}$$

$${}^*\mathrm{F} \xrightarrow{k_1} \mathrm{F} + h\upsilon_2 \text{ or } \Delta \tag{2}$$

$${}^*\mathrm{F} + \mathrm{Q} \xrightarrow{k_2} \mathrm{F} + \mathrm{Q} + \Delta \tag{3}$$

where F is the fluorophore in the electronic ground-state, $^*F$ is the fluorophore in the electronic excited-state, Q is the quencher, and Δ is the nonradiative decay energy emitted, usually as heat. k_1 is the first-order rate constant for decay of the excited-state, and k_2 is the sum of bimolecular rate constants for all decaying processes, including catalytic deactivation, energy transfer, and electron transfer.[19] Changes in fluorescence intensity can be calculated by the steady-state Stern−Volmer equation (eq 4).[20,21]

$$F_0/F_i = 1 + K_{SV}[\mathrm{Q}]_i \tag{4}$$

In this equation, F_0 is the initial fluorescence in the absence of quenching agent, F_i is the measured fluorescence in the presence of quenching agent, $[\mathrm{Q}]_i$ denotes the concentration of quenching agent, and K_{SV} corresponds to the Stern−Volmer quenching constant. This value is the slope from plotting the rate intensities against the quencher concentration when measuring the fluorescence in the presence of different concentrations of quencher, such as chloride.

Another deviation of the fluorescence is a nonlinear dependence of fluorescence intensity on fluorophore concentration.[22−25] This effect, known as IFE, significantly complicates the record of fluorescence excitation spectra and constants of fluorescence quenching by external quenchers, leading to incorrect uses of the technique.[23,24] Typically, the fluorescence intensity measured rises at first, reaches a maximum, and then decreases with increasing sample concentration.[23]

MATERIALS AND REACTANTS

Materials were selected on the basis of their availability at home or in most common stores. Details about the materials needed are listed below.

These materials are required to build the equipment:

- Excitation source, UV 275 nm excitation source such as a UV LED, UV pointer, or flashlight with a colored filter
- Smartphone with camera
- Small cardboard boxes
- Scissors
- Dark paint
- A cuvette (a polypropylene candy box can be used, such as a Tic Tac candy box)

For the experimental setup, the following materials are required:

- Fluorophore extracts
- Tonic water
- Alcohol 96% (or isopropyl alcohol)
- Distilled water
- Solvent (or paint thinner)
- Sodium chloride salt
- Sodium bicarbonate
- White vinegar (or lemon juice)
- Mixing bowls, a colander, and stirring utensils
- Paper or coffee filter

FLUOROMETER ASSEMBLY

Low-cost homemade equipment for fluorescence detection can be easily built using a cardboard box. The setup consists of three components: an excitation source, a sample holder, and a smartphone camera as fluorescence detector (Figure 1). The typical perpendicular geometry (90°) between the light source and the detector should be used for minimizing spurious signals from scattered and transmitted excitation. Distances between the excitation source, the sample, and the detector should be optimized to obtain reproducible measurements. The center of the camera must be focused on the center of the

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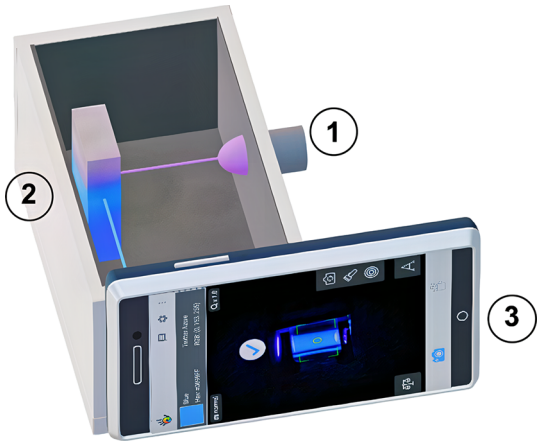

Figure 1. Diagram of the homemade device. Components: (1) excitation light source, (2) sample holder (polypropylene candy box), and (3) smartphone. The camera must be focused on the center of the sample holder. Note: In order to insert and place the UV LED, make a hole as small as possible and paste it with tape; no spurious light should enter.

sample holder (in this work, a polypropylene candy box has been used). The sample holder was placed around 7 cm from the detector and 5 cm from the excitation light source for a small box (e.g., 19 cm × 14.6 cm × 6.4 cm). According to the box size of each student, some adjustment might be required.

Flexibility is another aspect of the proposed homemade equipment. Most experiments require a UV light source for excitation, for example, a UV LED (275−295 nm) or a flashlight filtered with color optical filters.[26] These optical filters can be made using 5 layers of adhesive tape painted with permanent markers and using a flashlight as a white light source.[26] Also, the wavelength excitation is analyzed with blue, green, and red light from filtered light.

When image data is acquired by the smartphone camera, the output colors approach the eye sensitivity by decomposing in RGB channels (R = red, G = green, B = blue).[27] Each channel spans a certain range of wavelengths with peak sensitivity in the red, green, and blue regions of the visible light spectrum, and saturation refers to the color intensity. As the saturation increases, the colors appear to be increasingly pure. Value refers to the lightness or darkness of the color, where the darker areas will be represented with values close to 0 while the lighter areas have values close to 255.[28] Although the output colors on the smartphone screen do not directly reflect the detected light intensity, measurements are accurate enough to quantify fluorescence intensities and the quenching effect. These can be obtained using a color analysis application (*Color Grab* for Android, *Color Name* for iPhone, or similar) which senses essentially three-color spectrographs, R, G, and B (see SI for more information).[2,5,29]

So, in brief, the color of the extract solutions is observed by placing the cuvette in the sample spot of the homemade equipment, and the smartphone application is used to analyze the RGB pattern.

■ WET EXPERIMENTAL DETAILS

A brief description of each assay is written below, and a more detailed one is provided in the SI. Still, we encourage both instructors and students to participate in the construction of experiments designing alternatives based on their available materials. The solutions were prepared using a syringe for achieving more precision.

Reagent Preparation

Curcumin and chlorophyll extracts were obtained by mixing the natural material with 20 mL of 96% ethanol. After the mixture stood in the dark for 2−4 h, the extract was filtered and kept in the fridge until use. Chlorogenic acid was extracted with 96% ethanol from eggplant without peel for 30 min and filtered. This solution is very sensitive to oxygen, so in order to observe the fluorescence, the chlorogenic acid should be observed immediately after the extraction. Quinine samples were prepared by degassing commercial tonic water to avoid light dispersion caused by bubbles. This can be achieved by shaking the bottle, letting it rest, and then carefully opening it, or by pouring the sample in a glass and mixing with a spoon. Finally, the quencher solution (saturated NaCl solution) was obtained by dissolving 2 teaspoons in 20 mL of distilled water.

Effect of Solvent and pH on the Fluorescence Intensity

Following Table S1, aliquots of fluorophore extracts were mixed with some solutions in different vessels. The fluorescence was measured, and RGB values were analyzed.

IFE Assay

Five serial 1/2 dilutions of the fluorophores were made. Then, the fluorescence of each solution was measured with the homemade equipment. Finally, a plot of fluorescence intensity (intensity of R channel for chlorophyll or G channel for curcumin) vs fluorophore concentration (taking into account the relation between initial concentration, C_0) is generated.

Quenching Effect Assay

Tonic water was mixed with different concentrations of NaCl solutions following the experimental procedure depicted in Tables S2 and S3. Fluorescence was measured for each solution by triplicate, and the channel G value was registered (which corresponds to the F parameter in Stern−Volmer equation, eq 4). Then, F_0/F ratio was calculated (F_0 is the nonquenched sample, tonic water control sample) for each condition, and F_0/F vs the molar concentration of chloride was plotted.

■ HAZARDS AND SAFETY CAUTIONS

Heed the common laboratory safety regulations. However, ethanol 96% is flammable, mucosal irritating, or toxic if ingested. Vinegar can be irritating and should not be inhaled. Regarding the fluorometer, it is not recommended to stare directly at the excitation source without protection, such as sunglasses.

■ LOGISTICS AND SCHEDULE OF ACTIVITIES

We planned the experiments as a set of activities during social distancing due to the COVID-19 isolation. We tried to minimize the difficulties students may have during the performance in their home lab and, at the same time, ascertain the student capabilities to perform the experiments. The assays were presented to students divided into three sections:

(i) setting up the reagents and the material,
(ii) fluorometer assembly,
(iii) fluorescence and quenching experiments.

Throughout the experiments, if necessary, instructors could assist the students with their execution either by a video

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J. Chem. Educ. 2021, 98, 2625−2631

conference platform (Discord, Zoom, Jitsi, Meet) or by an instant chat application (Telegram, WhatsApp).

A useful likely schedule of activities is described in Table 1.

Table 1. Schedule of Activities

Week	Activities	Time Assigned, h
I	Selection of materials that students have at home	1−2
	Adjusting protocols to the available materials	1−2
	Preparing reagents: fluorophores extracts and solutions	4−6
II	Homemade fluorometer assembly	2−4
	Fluorescence and quenching measurement	2−4
III	Image and data analysis; report writing	1−2

RESULTS AND DISCUSSION

In the developed lab activity, different fluorophores solutions were tested: quinine (tonic water), curcumin (turmeric), chlorogenic acid (eggplant), and chlorophyll (spinach). The fluorescence of these compounds was observed irradiating the cuvette (or flask) in the homemade fluorometer and taking photographs with the smartphone (see Figure 1 and SI). When excited with UV light, these molecules display blue, green, yellow, and red fluorescence, respectively (Figure 2b−e). This

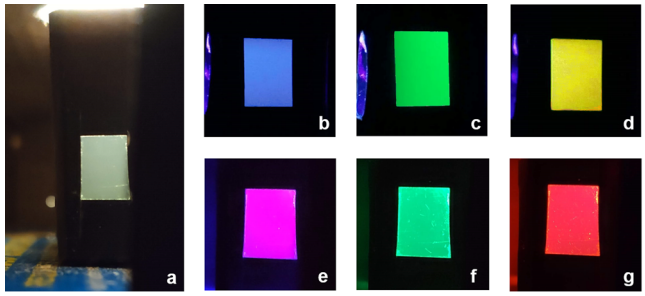

Figure 2. Pictures of (a) the sample holder with a mask, (b) quinine under UV excitation, (c) curcumin under violet excitation, and (d) chlorogenic acid under violet excitation. Chlorophyll under blue (e), green (f), and red (g) excitation. The excitation source wavelength (color) can be appreciated at the left of the mask (b−g).

simple experiment exemplifies that different emission is achieved despite all solutions being excited with the same wavelength. The excitation wavelength effect was evaluated on a chlorophyll sample using color filters and a flashlight, shown in Figure 2e−g. Only under UV light was an orange/red fluorescence observed. It is worth noticing that the eggplant extract (chlorogenic acid) decomposes very fast, so it was excluded from the protocols described here.

The light emission observed in these experiments provides substantial information for the introduction in class to the concept of the ground-state (S_0) and the singlet excited-state (S_1) of molecules. These are the fundamentals of the visible and well-discerned fluorescent phenomenon shown in Figure S1.

The fluorescence intensity also depends on the solvent used because it changes the polarity of the solvation environment,[30] and substances with acid−base properties modify their fluorescence with the pH of the medium. In this regard, the identification of the fluorescence intensity dependence on solvent and pH factors was studied by the students. Dilutions with different miscible solvents (water, thinner, and ethanol) were realized to analyze changes in the solvent, while the pH of the solutions was varied using acetic acid and sodium bicarbonate. Fluorescence variations were observed for curcumin and quinine solutions, as both compounds present a decrease in the fluorescence intensity when diluted in basic media,[31,32] whereas chlorophyll solution showed a diminution of the fluorescence intensity with acidic media since it decomposes[33] (the procedure is shown in the SI and results in Figure 3).

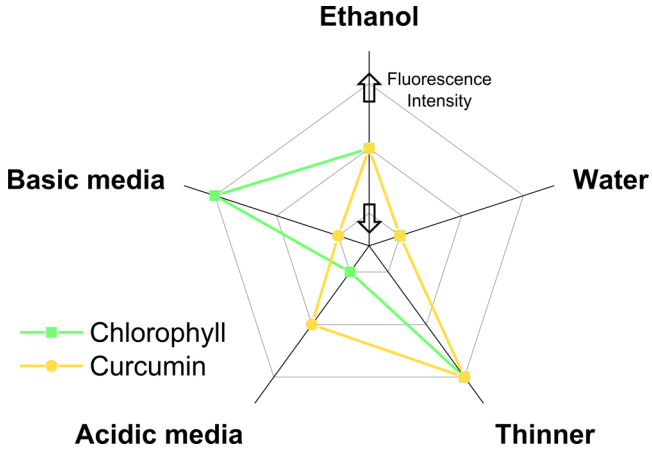

Figure 3. Qualitative variation of fluorescence when changing the solvent and the pH of the solution.

As we mentioned, quenching is a fundamental aspect of fluorescence. It refers to any process which decreases the fluorescence intensity of a given substance and has several applications such as imaging and sensing. This aspect is usually demonstrated by using quinine in the presence of chloride (sodium salt). For this purpose, the fluorescence intensity of degassed tonic water at room temperature was measured with the addition of solutions with different concentrations of sodium chloride. Quenched samples were prepared following the indications using different final NaCl molar concentrations; a Stern−Volmer plot was obtained and the K_{SV} calculated with a mean value of 125 ± 6 M^{-1} (Figure 4). This value agrees with the range presented in the literature, between 87 and 161 M^{-1}.[18,34]

On the other hand, IFE is the fluorescence phenomenon when the decrease in fluorescence emission is seen in concentrated solutions due to the absorption of exciting light by the fluorophore that is close to the incident beam and causing a significantly diminished light that reaches the sample further away from it. The conventional IFE correction gains importance in the analysis of optically dense fluorophore samples like oils, petrochemicals, biological samples, and food samples. IFE was studied by changing the fluorophore concentration using serial 1/2 dilutions of fluorophores. Results for chlorophyll and curcumin are depicted in Figure 5. Fluorescence intensity grows at first with the progressive increase in concentration (C/C_0 ratio is used instead, where C_0 is the initial fluorophore concentration in the extract), reaching a maximum and then decreasing. The experimental data obtained had a good correlation with the dependence of the total fluorescence intensity at high concentrations of fluorophore caused by this effect,[22−25] proving to be an appropriate experimental setup to demonstrate this behavior.

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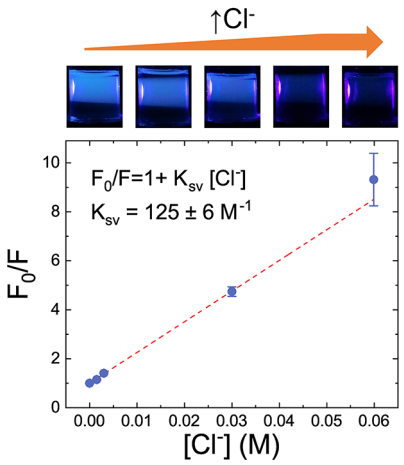

Figure 4. Pictures of quinine samples with different concentrations of NaCl (top). Stern–Volmer plot for the quenching of quinine fluorescence by NaCl (bottom).

For all the work presented here, it must be kept in mind that the color intensity might differ from one student's assay to another: Not every tonic water brand contains the same concentration of quinine, nor are the chlorophyll or curcumin extracts always the same. Also, RGB values differ for each individual student, depending on their specific fluorometer configuration and smartphone, for example, the camera hardware, the excitation source spectrum, and the geometrical distances and alignment. Nonetheless, the results obtained proved to be robust and self-consistent, and all the proposed concepts can be experimentally recognized by the students.

STUDENT OUTCOME

Two different strategies were used to evaluate and measure the experience of the students. First, students carried out the practical experience and answered the same questions before carrying out the practical experience (pretest) and after its completion (posttest). These questions are presented in Table S4. In this way, we intended to compare the results obtained in the posttest with those of the pretest to analyze the degree of learning (Figure 6A). We noticed that there was a noticeable increase in the correct answers after performing the hands-on experiment at home.

Second, an inquiry was made after the student finished the activity where they chose the option that best suits them regarding the statements listed in Table S5. The experimental work and the activity went smoothly, and the students described it as straightforward and easy to understand. Figure 6 B depicts some of the statements they made. They commented that the activity was helpful because it visually demonstrated the named concepts. Moreover, the enjoyable enthusiasm of the students when seeing the beautiful emitted light of the molecules cannot be put aside, as they were tasked with formulating their own laboratory tools, stimulating their curiosity.

In summary, this activity gave the students an opportunity to put into action the scientific method: several questions have to be answered; the way students will build the equipment and perform the measurements must be chosen in a challenging mode; data measurement is collected and should be organized and plotted; conclusions and comparisons should be made. Table S6 summarizes the main points and concepts that were discussed during the performance of the proposed hands-on experiment. As a final result, students developed manual, observational, inquisitive, and investigative skills that range from planning experiments to interpreting data, while learning scientific concepts and facing cognitive organizational and communicative challenges.

CONCLUSION

Teaching fluorescence spectroscopy aims at giving students a deep understanding of light–matter interactions. In this work, we described a simple, easily built, inexpensive, and homemade fluorescence method using a UV excitation source and a smartphone camera with an RGB analysis application as the detector. In this way, students became involved in the data analysis by using smartphone apps, which are innovative as laboratory tools. The experiments combine science and a device familiar to them, increasing their interest and motivation for learning. Because these assays are safe to

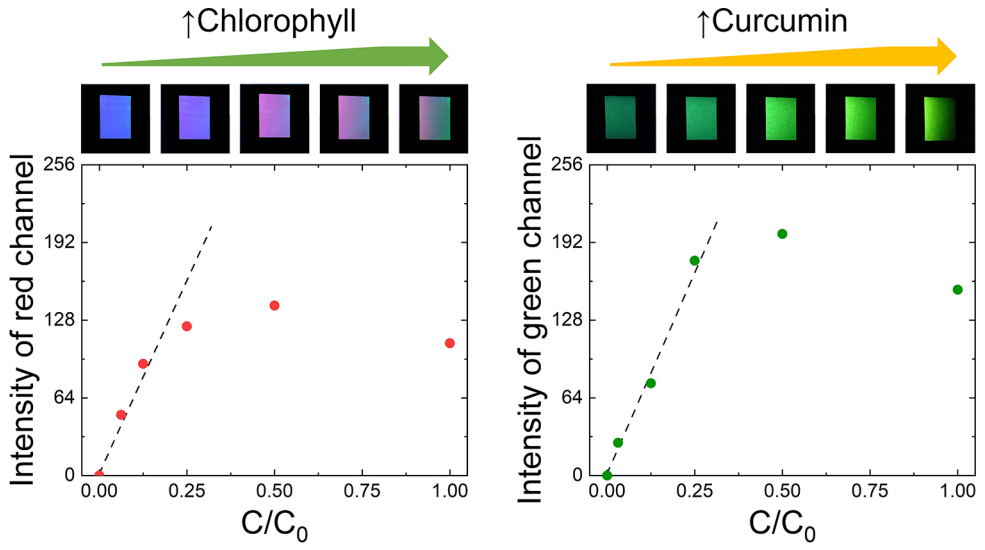

Figure 5. Pictures of samples with different concentrations of fluorophore (top). Fluorescence intensity as intensity of RGB channel vs fluorophore C/C_0 ratio (bottom). C_0: initial fluorophore concentration in the extract.

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J. Chem. Educ. 2021, 98, 2625–2631

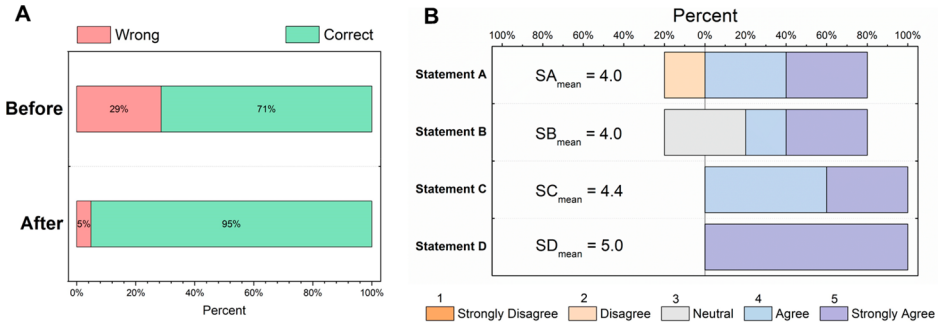

Figure 6. (A) Questionnaire results before (pretest) and after (posttest) and (B) inquiry results for statements: A, Assembling the device was easy for me; B, The materials to perform the activity were easy to get; C, The activities helped me to understand the subject more clearly; D, The activities made me curious to do other experiments at home.

perform and the bright colorful emission can be observed with the naked eye before using the smartphone, they can be used to teach students of different ages, backgrounds, and diverse educational levels. Likewise, the instructor can use this activity as a class demonstration by recording a video, and students are able to complete the experiments with careful online guidance and demonstrate successful outcomes through discussions and formal reports.

These laboratory experiments were implemented as a home experience for overcoming the disruption of teaching activities which came along with the social isolation during the COVID-19 pandemic.[35,36] We consider these points highly relevant, given that remote education might aggravate pre-existing social inequalities and quality of education. These experiments also provided a unique alternative to teach and measure fluorescence when no fluorometer or equipment is affordable, even in face-to-face education. In addition, if no smartphone is available, students can use the hands-on experiment and evaluate the changes in fluorescence qualitatively by making comparisons between the prepared solutions. Furthermore, we believe remote education to be an opportunity to develop better teaching materials through inventiveness to assist the development of learning skills.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available at https://pubs.acs.org/doi/10.1021/acs.jchemed.1c00328.

> Details regarding the Jablonski diagram, materials and reagents, methodology, directions for building the equipment for fluorescence detection, fluorophore extraction preparation, extract fluorescence measurements, identifying the effect of the physicochemical environment, inner filter effect assay, quenching effect assay, questions and answers for the pre- and postlab multiple-choice quiz, questionnaire statements for students after the activity, and main points and concepts that were discussed during the assays (PDF, DOCX)

■ AUTHOR INFORMATION

Corresponding Authors

Diego Onna − *Instituto de Nanosistemas, Universidad Nacional de San Martín (INS-UNSAM), San Martín CP 1650 Buenos Aires, Argentina; Departamento de Química Inorgánica, Analítica y Química Física, Facultad de Ciencias Exactas y Naturales, Universidad de Buenos Aires, C1428EGA Buenos Aires, Argentina;* orcid.org/0000-0002-3158-1915; Email: diego.onna@qi.fcen.uba.ar

Mariana Hamer − *Instituto de Nanosistemas, Universidad Nacional de San Martín (INS-UNSAM), San Martín CP 1650 Buenos Aires, Argentina;* orcid.org/0000-0002-2391-2913; Email: mhamer@unsam.edu.ar

Authors

Micaela Hamer − *Instituto de Patobiología Veterinaria (IPVET), INTA-CONICET, Hurlingham CP 1686 Buenos Aires, Argentina;* orcid.org/0000-0003-3361-1732

Agustina Maria Beraldi − *Laboratorio de Investigación y Desarrollo en Ingeniería Química (LIDIQ), Universidad Tecnológica Nacional Facultad Regional Avellaneda (UTN FRA), Avellaneda B1874ABY Buenos Aires, Argentina; Instituto de Nanosistemas, Universidad Nacional de San Martín (INS-UNSAM), San Martín CP 1650 Buenos Aires, Argentina;* orcid.org/0000-0002-1813-9567

Sergio G. J. Gomez − *Instituto de Nanosistemas, Universidad Nacional de San Martín (INS-UNSAM), San Martín CP 1650 Buenos Aires, Argentina;* orcid.org/0000-0001-9194-5664

Facundo Ortega − *Instituto de Patobiología Veterinaria (IPVET), INTA-CONICET, Hurlingham CP 1686 Buenos Aires, Argentina;* orcid.org/0000-0002-3815-1264

Complete contact information is available at: https://pubs.acs.org/10.1021/acs.jchemed.1c00328

Notes

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