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Demonstration

Easy, Bright, Fluorescence Demonstration of Buffer Action

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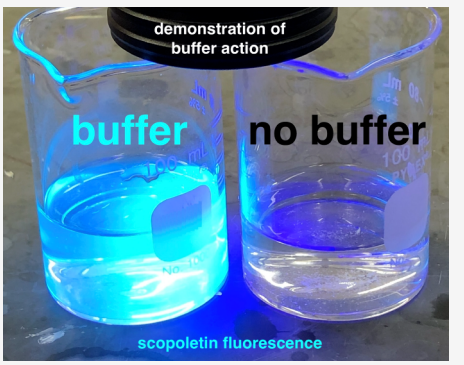
ABSTRACT: We report an attractive update to the demonstration of acid−base buffer action that is appropriate for ease of use and getting attention in the classroom. The twist is based on new fluorophore information and the recent availability of UV flashlights serving as an ideal, portable excitation light source. The pH-dependent fluorescence comes from a choice among three natural sources, namely narra tree wood extract, kidneywood extract, and scopoletin, which is a purchasable coumarin, that all perform equally well. We provide practical details for performing the buffer demonstration at scales of 100 and 1000 mL and give background information for context.



KEYWORDS: *High School/Introductory Chemistry, First-Year Undergraduate/General Chemistry, Acids/Bases, Buffers, Fluorescent Indicator, pH, Chemical Demonstration*

We describe a novel approach to demonstrating acid−base buffer action using an eye-catching fluorescent indicator. The use of colored pH indicators to show buffer action has been done effectively for many years.[1,2] A small amount of acid or base induces a significant color change in an unbuffered solution, whereas a buffered solution requires significantly more volume addition before a change is observed. Our scheme depends on a pH change that turns off a bright fluorescence that is eye-catching in normal room light. The success of this scheme relies on an optimal match between the excitation source using an easily available ultraviolet light-emitting diode (UV LED) flashlight and a choice of several pH-dependent fluorophores that exhibit high fluorescence efficiency (quantum yield), namely narra wood aqueous extract, kidneywood aqueous extract, and a coumarin called scopoletin. The narra tree (*Pterocarpus indicus*, which is the national tree of the Philippines) and kidneywood (*Eysenhardtia polystachya*, from Mexico) have wood that easily produces a highly fluorescent compound in alkaline water that was identified as matlaline in 2009;[3] the pH-dependent fluorescence of narra extract has been previously described in this *Journal*.[4−6] A scopoletin solution can also be obtained as an aqueous extract from London planetree (sycamore tree) wood, a process that has also been described in this *Journal*.[7] However, unlike the narra wood and kidneywood solutions, scopoletin is available as a pure compound. The portability of the UV LED flashlight with its excellent match to the photophysical characteristics of these fluorophores makes this a very attractive choice for the chemical demonstration of buffer action. We describe here the details for carrying out this demo on two scales (100 mL and 1 L), an opportunity to illustrate the Henderson−Hasselbalch relationship, and some background information on fluorescence.

BUFFER DEMONSTRATION DESCRIPTION

General Description of Demonstration with Narra Tree Fluorophore

A narra stock solution was diluted by a factor of 10 in either deionized water or a 7.5 pH buffer (0.1 M). A small addition of base ensured the pH of the unbuffered solution was above 7.5. These two solutions were illuminated by the UV flashlight to show that they glowed a similar bright blue, easily visible in normal room light. The buffered narra solution can be prepared ahead of the demonstration, although it adds impact to the demonstration to have premeasured amounts of the weak acid and conjugate base to combine during the demonstration to show the creation of the buffer using a volume ratio that illustrates the Henderson−Hasselbalch relationship. The learning objective of the demo is to see that the presence of a buffer limits the change in pH. Adding a small amount of acid to the unbuffered solution while stirring it dramatically shuts down the fluorescence, while a similar addition of acid to the buffered solution has no visible impact on the bright blue glow. Several more additions have no effect

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as well, thus illustrating a significant difference due to the buffer limiting the change in pH. With appropriate proportions, enough acid can be added to illustrate a limit to the buffer capacity. This can be the extent of the demonstration, but it can also be shown that adding base can restore the fluorescence to either of the two solutions. As an alternate to narra, a kidneywood aqueous extract can be used, as it performs similarly with the same bright blue fluorescence; the fluorophore and the pH of the buffer are the same.

Alternate Demonstration with the Scopoletin Fluorophore

The demonstration using a scopoletin solution is quite like the wood extract versions. The difference here is that scopoletin is a compound that can be purchased as a pure substance, and it is buffered at pH = 9.2. A later section provides details specifying the amounts needed to perform the demonstration on scales of 100 mL and 1 L.

■ DEMO USE IN THE CLASSROOM

While this demonstration will fit well into any chemical demonstration show with an emphasis on acid−base concepts or fluorescence/luminescence, its inspiration is as a brief, but effective, classroom demonstration in the context of acid−base buffers, a topic that is normally covered in college-level introductory chemistry but is also encountered in AP chemistry and other courses. Its use at our institution was after a presentation of the Henderson−Hasselbalch equation, which establishes how a ratio of weak acid and conjugate base form a pH buffer relative to the pK_a of the buffer system. The narra demo was used by one of the authors (M.M.) in two sections of 30−40 students in a general chemistry II course (university level, major's course) and by a colleague in similar fashion in a third section. The demonstration can be made much more elaborate to include the measurement of the pH, but part of the appeal of this demonstration is that it can be kept quite simple, using class time efficiently.

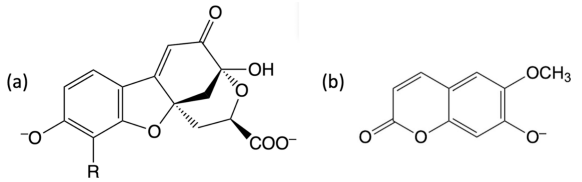
The demonstration described here offers a significant advantage over the more traditional color-changing indicator because the change is manifested by eye-catching light emission from a brightly glowing beaker rather than just light absorption producing a color.

■ FLUORESCENCE BACKGROUND INFORMATION

Description of the Fluorophores

Scheme 1 shows the structures of the fluorophores matlaline, found in the narra/kidneywood extracts, and scopoletin.

Scheme 1. Fluorescent pH Indicators (a) Matlaline Dianion (R = C-Glycosyl Group) and (b) Scopoletin Anion[a]



[a]Both indicators are shown in the high pH ionized form, where their 395 nm-excited fluorescence is brightest.

Description of the Scopoletin Fluorophore

The fluorescence of scopoletin has been recently described by Pina et al.[8] and Pham et al.[9] It has been identified as the primary fluorescent compound in sycamore tree aqueous extract,[7] where its role is as a bioactive, secondary metabolite produced in response to plant stress. Figure 1 shows that the absorbance spectrum of scopoletin depends on pH. Good overlap between the absorbance of scopoletin at pH = 9 (Figure 1, trace B) and the emission spectrum for the 395 nm flashlight (Figure 1, trace C) gives a strong, blue fluorescence emission peak at 465 nm (Figure 1, trace D). The blue-shift in the absorbance when the pH is lowered below the pK_a (7.3) causes the overlap with the LED emission of the UV flashlight to be 50 times less at pH = 5 compared to at pH = 9. The fluorescence efficiency is only modestly impacted by pH.[9] Thus, the primary effect of reducing the pH in scopoletin solution is the dramatic change inthe absorbance spectrum shown in Figure 1 such that scopoletin no longer significantly absorbs the excitation light. This absorbance shift is the heart of the matter when we observe color changes in traditional acid−base indicators. Solution absorbance at a concentration of 10 μM and pH = 9 is <0.2 near the peak wavelength of the UV flashlight emission, allowing the excitation light to penetrate deep into the solution and produce emission from nearly the entire volume of liquid, which is a key to the visual impact. The inset in Figure 1 shows that a pH around 9 is an optimal choice for the buffer demo because this is where scopoletin is nearly completely in its anion form; in contrast, lowering the pH by 3 units converts it to its far-lower absorbing, protonated, neutral form.

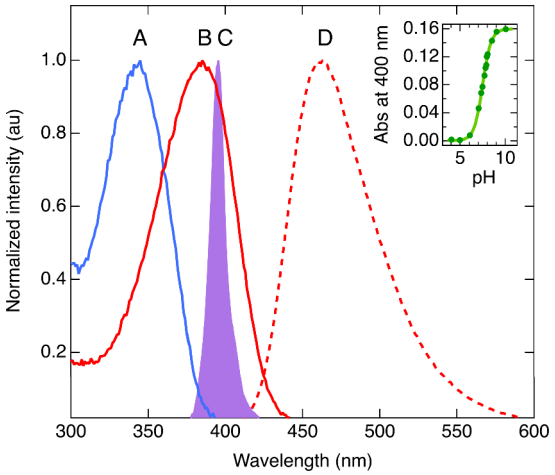


Figure 1. Normalized absorbance and emission spectra for scopoletin above and below its pK_a (7.3). (A) Absorbance at pH = 5 (solid, blue trace). (B) Absorbance at pH = 9 (solid, red trace). (C) Emission from the 395 nm LED flashlight (fill-to-zero, violet trace). (D) Emission of scopoletin at pH = 9 (dashed, red trace). The inset shows the change in scopoletin absorbance at 400 nm, near the peak of the UV flashlight, indicating that the transformation from acid to conjugate base occurs over the pH range of 6−9.

While scopoletin is not usually found in a list of fluorescent acid−base indicators, it is in the class of compounds called coumarins and is closely related to the known coumarin fluorescent indicators aesculetin and umbelliferone, which are described in Sabnis's handbook on acid−base indicators.[10] Compared to aesculetin and umbelliferone, scopoletin is

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J. Chem. Educ. 2024, 101, 701−705

ideally matched for this demonstration based on its high quantum efficiency[9] and its absorbance characteristics relative to the 395 nm excitation.

Description of the Narra Tree and Kidneywood Fluorophore

The fluorescent compound in narra wood and kidneywood extracts has been identified only recently by Acuña et al.,[3] even though the history of this spectacular fluorescence goes back nearly 450 years to its earliest-known written account when the wood was called *Lignum nephriticum*, which is Latin for "kidney wood". The name ties to the description of its medicinal use in the 16th century, and it is interesting to note that the tea made from Mexican kidneywood, also known as Palo Azul (blue stick), is still said to promote kidney health (a claim mentioned by most websites that promote Palo Azul but also by an academic website on herbal safety[11] and a peer-reviewed study on the genus *Eysenhardtia*[12]). The blue fluorescent phenomenon received the scrutiny of Isaac Newton, Robert Boyle, and George Stokes, and its history[13] intertwines two trees from places that are nearly antipodes on the planet, Mexico (kidneywood, *E. polystachya*) and the Philippines (narra, *P. indicus*)—the trees stem from the same plant family, Fabaceae. Jameson[14] pointed out in his recent textbook on fluorescence that Boyle first described the pH dependence of *L. nephriticum* in 1663, so this demonstration that applies the phenomenon to demonstrate buffer action makes it accessible 360 years later! Obtaining the fluorescent extract from narra tree wood has been described by Muyskens[4,5] and adapted for this demonstration. The extract transition from dark to bright fluorescence occurs over the pH range of 5−7, as shown in Figure 1 of ref 4. The efficiency (quantum yield) of the narra and kidneywood fluorophore at high pH is 100%,[3] which is the key to the success of this demonstration: the brightness and color are striking without dimming the room light. Acuña et al.[3] indicated that the absorbance at ∼400 nm is relatively unchanged with pH, but the low pH form of matlaline is nonfluorescent. Thus, when matlaline dianion becomes protonated, it loses its highly fluorescent character. This offers a notable contrast with the pH effect on scopoletin fluorescence, which is due primarily to the change in absorbance.

DEMONSTRATION DETAILS

Buffer Demonstration with Narra Extract

Preparation of the stock solution requires obtaining a piece of *P. indicus* wood and turning it into shavings. The easiest, most low-cost approach is to find a vendor of an Amboyna burl pen blank (a small block of wood, typically 3/4″ × 3/4″ × 5″); in the best circumstances, the wood will be identified by the vendor as *P. indicus*. The amount of stock solution that is needed depends on the scale of the demonstration. To prepare for several demonstrations on the 100 mL scale, 1 g of wood shavings was stirred into 55 mL of water with a couple drops of base (∼0.05 M). Then, the wood was steeped for about 5 min at room temperature, and the solution was either decanted or filtered to remove the shavings to produce about 50 mL of stock solution. This yellow solution was later diluted by 10× for the demonstration. Representative narra photos are included in the Supporting Information.

Narra wood fluorescent extract buffered to about pH = 7.5 held the pH above the pK_a for the fluorescent form of the fluorophore in the demonstration. Using a 0.1 M buffer system based on $H_2PO_4^-/HPO_4^{2-}$ (pK_a = 7.21), a ratio of conjugate base to weak acid of 2:1 using the Henderson−Hasselbalch equation gives a pH of 7.5. It is convenient to make this ratio a part of the demonstration because volumes of the two buffer components can be poured together, diluting the narra stock solution by 10×; for example, with a final volume of 100 mL, 30 mL of $H_2PO_4^-$ and 60 mL of HPO_4^{2-} can be mixed with 10 mL of the stock solution. While this outline is sufficient for guidance, please refer to the Supporting Information for more details.

Buffer Demonstration with Kidneywood Extract

Preparation of the stock solution requires obtaining *E. polystachya* wood. This is straightforward, as one can do an Internet search for a vendor of Palo Azul, which comes in the form of either wood chips or finer wood pieces contained in a teabag: both kinds work equally well for this demo. About 12 g of chips in 200 mL of room-temperature water produced a good stock solution in 10 min; using the teabag took less time. Alkaline water is recommended by some Palo Azul suppliers, and this produced a dark solution, although natural spring water (e.g., Evian) and tap water (Grand Rapids, Michigan, U.S.) also worked well; in contrast, deionized water was poorly fluorescent during steeping, although adding base afterward produced good fluorescence. The dilution factor for the stock solution depends on its concentration, which depends strongly on the form of the wood, the type of water used, and steeping time. Wood chips, as described above, in natural spring water produced a yellow solution that worked well with a factor of 10 dilution, like the narra stock solution described above. The teabag (∼2.2 g) steeped in 200 mL of tap water for 10 min at room temperature produced a darker stock solution than the wood chips; 3−4 mL of this stock solution diluted to 100 mL of DI water produced a good unbuffered solution for the demo. The goal is to obtain a solution that is as bright as possible that is also still uniformly illuminated throughout the volume; a solution that is too concentrated will be brighter near the bottom when lit from below. Kidneywood buffering considerations are the same as those for the narra extract.

Buffer Demonstration with Scopoletin

Preparation of the stock solution requires obtaining scopoletin; we describe using the pure compound rather than following the procedure by Wharton et al.[7] to make sycamore wood extract. Many vendors supply scopoletin (6-methoxy-7-hydroxycoumarin, CAS No. 92-61-5) with widely varying prices per gram. Only 20 mg is needed to prepare a 100 mL volume of a 1 mM stock solution, which can serve as the stock solution for 100 demos on the 100 mL scale or 10 demos on the 1 L scale. At the time of this writing, the INDOFINE Chemical Company (Hillsborough, New Jersey 08844, U.S.) had the lowest price per gram, and the company indicated they were willing to sell 100 mg quantities for under $50. Scopoletin was buffered at around pH = 9.2 to hold it above its pK_a. Using a 0.1 M buffer system based on HCO_3^-/CO_3^{2-} (pK_a = 10.25), we needed a ratio of conjugate base to weak acid of 1:10. Like the previous case, this is a volume ratio that can be highlighted as part of the demonstration.

The choice of fluorophore for this demonstration centers on the preference to obtain the fluorescent stock solution from wood (narra or Palo Azul) or from a purchased compound (scopoletin) and whether a historical or botanical significance plays a role in your choice.

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J. Chem. Educ. 2024, 101, 701−705

Other Fluorophore Notes

The highlighted fluorophore/excitation choices are influenced by the expertise of the authors. It is possible to use the fluorescence of other pH-dependent fluorophores, offering potentially a more visibly evident Stokes shift. However, other candidate compounds such as fluorescein, for example, are not in our area of research, and multi-LED, large-aperture flashlights are not necessarily available in a color ideally suited for excitation. However, the principles used in this demonstration description can be applied to adapt them to other fluorophores.

Excitation Light Source Considerations

A key feature of the demonstration is the availability of an inexpensive, wide-aperture UV flashlight. A common version has a 51-LED array powered by three AA batteries, which makes the excitation light source compact and portable. This size fits a typical 100 mL beaker for very uniform illumination from underneath the beaker, whereas the 68-LED version works well under a 150 mL beaker. An effective way to scale up the demonstration to the 1 L scale is to position three UV flashlights equally around a 2 L beaker to uniformly illuminate it; see the Supporting Information for more details. UV flashlights are popular and readily available due to their applications in detecting fluorescence in household stains and official documents, such as driver's licenses and passports. Other "UV LED blacklight" configurations include bars and ribbons. LED flashlights in other colors are available, but the choice of color and flashlight diameter/number of LEDs is quite limited.

SAFETY CONSIDERATIONS

Hazards associated with the handling of liquids for this demo involve irritants and corrosive substances that warrant limiting exposure to skin and eyes. Gloves are recommended for handling the acids and bases at the concentrations needed for the 1 L demonstration. Secondary containment for the acid and base volumes is also recommended at these concentrations to mitigate any unanticipated spills while handling them and performing the demonstration. The Safety Data Sheet for scopoletin (CAS No. 92-61-5) indicates that while this compound is not known to be a hazardous substance, its toxicological properties have not been thoroughly investigated. The narra stock solution should be considered a liquid with unknown hazard properties and treated with due caution. The kidneywood solution is advertised as a drinkable tea and can be considered a low-hazard substance within the normal expectations of handling substances in a chemistry lab. Disposal of the liquids should be consistent with local regulations and policies for handling waste materials. The Shakhashiri text[2] offers some advice regarding the hazards and disposal associated with buffer demonstrations.

UV flashlights should be handled with the same caution as for light sources that have significant emission outside the visible spectral region. Steps include minimizing eye exposure, for example, by not shining the beam directly into a person's eyes for a prolonged period. Emission spectra indicate that polycarbonate lab safety glasses' lenses will reduce the emission from the 395 nm flashlight by over 50% by absorbing mainly the shorter wavelength portion of the emission spectrum. With these general safety precautions in mind, the demonstration process described here involves directing the UV flashlight at the desired solutions for only as long as is needed to show emission, and thus, wide exposure to UV light is reasonably contained and minimized.

ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available at https://pubs.acs.org/doi/10.1021/acs.jchemed.3c00792.

> Tips for practitioners of the demo, illustrative photos of narra demonstration, details for performing the demo at the 50 mL scale, a link to a video of the 1 L-scale demonstration, and notes for the 1 L-scale demonstration (PDF, DOCX)

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Notes

The authors declare no competing financial interest.

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REFERENCES

(1) American Chemical Society − Chemistry in Context. Buffer Demonstration. https://www.acs.org/education/resources/undergraduate/chemistryincontext/interactives/health-and-medicine/buffer-demonstration.html (accessed 2024-01-28).

(2) Shakhashiri, B. In *Chemical Demonstrations, Vol. 3: A Handbook for Teachers of Chemistry*; The University of Wisconsin Press, 1989; Vol. 3.

(3) Acuña, A. U.; Amat-Guerri, F.; Morcillo, P.; Liras, M.; Rodríguez, B. Structure and Formation of the Fluorescent Compound of Lignum Nephriticum. *Org. Lett.* **2009**, *11* (14), 3020−3023.

(4) Muyskens, M.; Vitz, E. The Fluorescence of Lignum Nephriticum: A Flash Back to the Past and a Simple Demonstration of Natural Substance Fluorescence. *J. Chem. Educ.* **2006**, *83* (5), 765.

(5) Muyskens, M. A. pHantastic Fluorescence. *J. Chem. Educ.* **2006**, *83* (5), 768A−769A.

(6) Acuña, A. U. More Thoughts on the Narra Tree Fluorescence. *J. Chem. Educ.* **2007**, *84* (2), 231.
(7) Wharton, J.; Izaguirre, I.; Surdock, A.; VandenBerg, M.; Bolhuis, S.; Howard, J.; Muyskens, M. Hands-On Demonstration of Natural Substance Fluorescence in Simple Tree Extracts: Sycamore. *J. Chem. Educ.* **2018**, *95* (4), 615−619.
(8) Pina, J.; de Castro, C. S.; Delgado-Pinar, E.; Seixas de Melo, J. S. Characterization of 4-Methylesculetin and of Its Mono- and Di-Methoxylated Derivatives in Water and Organic Solvents in Its Ground, Singlet and Triplet Excited States. *J. Mol. Liq.* **2019**, *278*, 616−626.
(9) Pham, H. T.; Yoo, J.; VandenBerg, M.; Muyskens, M. A. Fluorescence of Scopoletin Including Its Photoacidity and Large Stokes Shift. *J. Fluoresc* **2020**, *30* (1), 71−80.
(10) Sabnis, R. W. In *Handbook of Acid-Base Indicators*; CRC Press, Boca Raton Florida, U.S., 2007. DOI: 10.1201/9780849382192.
(11) Stuart, A. G. Herbal Safety: Kidneywood. https://www.utep.edu/herbal-safety/herbal-facts/herbal%20facts%20sheet/kidneywood.html (accessed 2024-01-28).
(12) Garcia-Campoy, A.; Garcia, E.; Muñiz-Ramirez, A. Phytochemical and Pharmacological Study of the Eysenhardtia Genus. *Plants (Basel)* **2020**, *9* (9), 1124.
(13) Safford, W. E. In *Lignum Nephriticum: Its History and an Account of the Remarkable Fluorescence of Its Infusion*; Smithsonian Institution, 1916.
(14) Jameson, D. M. In *Introduction to Fluorescence*; CRC Press, Boca Raton, Florida, U.S., 2015. DOI: 10.1201/b16502.

https://doi.org/10.1021/acs.jchemed.3c00792
J. Chem. Educ. 2024, 101, 701−705