

Using Luminescence To Show Intramolecular and Intermolecular Hydrogen Bonding: An Activity for General Chemistry or Physical Chemistry

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Supporting Information

ABSTRACT: A hands-on activity for students in general chemistry and physical chemistry courses has been designed to facilitate study of intramolecular (IntraHB) and intermolecular (InterHB) hydrogen bonding in biomolecules using luminescence. 3-Hydroxyflavone (3-HF), the backbone of all flavonols, is a promising model molecule, for it possesses an excited-state intramolecular proton transfer (ESIPT) effect that can serve as a fluorescent probe for studying many biological systems. The luminescence of 3-HF has one or two bands, whose intensities are dependent on temperature and solvent. In this activity, molecular models are used to illustrate hydrogen bonding of 3-HF in the ground state in two solvents, ethanol and hexane. Then students perform an activity to enable them to observe the luminescence of 3-HF in ethanol and in hexane, at room temperature and at 77 K, respectively, followed by discussion of the luminescence of 3-HF in ordinary filter paper. The project allows students to relate the idea of hydrogen bonding in the ground state to a luminescence color change in the excited state through a naked-eye experiment.



KEYWORDS: First-Year Undergraduate/General, Upper-Division Undergraduate, Hands-On Learning/Manipulatives, Hydrogen Bonding, Dyes/Pigments, Fluorescence Spectroscopy, Graduate Education/Research

Hydrogen bond interactions play a very important role in biological and chemical systems.¹ It is commonly explained in many general chemistry texts and experiments. The topics include high boiling points, high melting points, and high heat of fusion of water.² Hydrogen bonding (HB) refers to the interaction that occurs between a hydrogen atom (hydrogen donor) bonded to a highly electronegative atom such as N, O, F and another electronegative atom (acceptor) with lone pairs on a different molecule or chemical group.^{3,4} Hydrogen bonds may be either intramolecular (IntraHB) or intermolecular (InterHB).³ For IntraHB to occur, both a hydrogen donor and acceptor moieties must be located in close proximity to each other in the same molecule. For InterHB to occur between one molecule and a neighboring molecule, the hydrogen donor and acceptor must be positioned such that they can interact.⁵ Since the structures of biomolecules are partly stabilized by IntraHB and InterHB, hydrogen bonds play a pivotal role in aspects such as molecular recognition, mechanical strength, and binding specificity.^{6,7}

The phenomenon of HB in the ground state has been investigated extensively by experimental and theoretical methods.⁸ For a molecule which contains an IntraHB, upon photoexcitation, the strength of HB increases, which in turn increases the acidity/basicity of the hydrogen donor and acceptor.⁸ For example, on the basis of previous calculations,⁹ the lowest singlet excited state of 3-hydroxyflavone (3-HF) corresponds to the HOMO–LUMO π – π * electronic transition. Accordingly, for a molecule which contains an IntraHB, like 3-HF, in its chemical structure, on photoexcitation, there

must occur an electron density redistribution, and simultaneous substantial increase, in the acidity of the proton donor and the basicity of the proton acceptor.^{10,11} Subsequently, a hydrogen (also called a proton) migrates from an acidic moiety to a basic moiety within close proximity to each other via excited-state intramolecular proton transfer (ESIPT).⁸ Owing to their importance in serving as model systems in chemistry and life sciences, excited-state proton transfer molecules have attracted considerable attention.¹² Among these molecules, 3-HF (see Scheme 1) is a prototype.^{13–15}

The ESIPT process of 3-HF is illustrated in Scheme 1. Upon excitation of 3-HF, the phenolic proton of the excited normal form converts to the opposite heteroatom to form the excited tautomer form.¹³ Eventually, the excited normal and tautomer structures decay to the ground state by emitting a dual luminescence,¹⁴ including normal emission (F(Normal)) and tautomer emission (F(Tautomer)), respectively. In the ESIPT process, the geometric structure of the excited tautomer form is significantly different from that of the ground state normal form. As a result, the second luminescence band has a large Stokes shift relative to the first one.¹⁵ F(Normal) and F(Tautomer) luminescence intensities are sensitive to temperature and solvent properties. The exact wavelengths of F(Normal) and F(Tautomer) are strongly dependent on the interaction with the environment.⁹



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Scheme 1. Schematic Energy Level Diagram for ESIPT in 3- HF^a



^{*a*}Asterisk (*) in Schemes 1-4 denote the excited state.

Several papers on IntraHB and/or InterHB interactions have been published. For instance, Schultz et al.¹⁶ demonstrated that using models and demonstrations for teaching chemistry can help make science content accessible and understandable to the learner. In another journal, Hammer¹⁷ and Douglas et al.¹⁸ described luminescence as a fun and exciting tool for teaching basic science concepts. Although the above works have focused on developing experiments, models, and ideas for teaching HB, to our knowledge, relatively few reports have focused on using luminescence to describe HB.

Bearing all of this in mind, in this hands-on activity, molecular models are combined with simple experiments to illustrate IntraHB and InterHB in different solvents through luminescence. First, molecular models are used to illustrate the geometry of 3-HF in the ground state in different solvents. Then students observe the luminescence of 3-HF in ethanol and in hexane, at room temperature (RT) and at 77 K, respectively. Students then try to identify which luminescence color from the excited state is due to IntraHB or InterHB in the ground state. Molecular models can help teachers explain concepts of HB and ESIPT. Finally, the luminescence of HB of 3-HF in ordinary filter paper is examined as an extended study of the HB idea. This procedure is suitable for students in general chemistry and physical chemistry courses, and the entire activity requires about 3 h for general chemistry and 2 h for physical chemistry.

PROCEDURE

Part 1: Building Molecular Models of 3-HF in Ethanol and in Hexane

At the start of the student activity, the class is divided into teams of 2–4 students. Four standard-size student desks are arranged together as an activity space for each team. The teacher explains the concepts of HB, IntraHB, and InterHB between molecules and solvents on a blackboard first. The students of each team try to build 3-HF molecules with ethanol or hexane using ball-and-stick molecular model kits. Building molecular models reinforces students learning regarding molecular structure, and by relative placement of 3-HF and solvent molecules, students can explore possible HB interactions (Figure 1a,b).

Part 2: The Luminescence of 3-HF in Ethanol and in Hexane, at RT and 77 K

Activity

At first, the instructors explain the definition of luminescence to the students (see the Supporting Information). And the stock solution $(2 \times 10^{-3} \text{ M})$ of 3-HF is prepared first in the lab to simplify the activity by the instructors. The solution of 3-HF $(2.0 \times 10^{-5} \text{ M})$ is prepared using spectroscopic-grade ethanol (Sigma-Aldrich, >99.8%) and hexane (Sigma-Aldrich, \geq 95%), respectively. Glass tubes are filled with 5.0 mL of the solution of 3-HF in the respective solvents. At this time, the instructors turn off the lights and keep the room dark. The solutions are exposed to a standard hand-held UV ($\lambda = 365$ nm) lamp (Figure 2a,b). Each team tries to figure out why the luminescence differs in ethanol and in hexane. The instructors can help students draw these two different luminescence pathways with the assistance of the molecular models in Part 1, so that team members can relate the color of the luminescence to the HB interaction in the ground state.

When the students understand the difference in luminescence of 3-HF in different solvents, they place the two glass tubes in liquid nitrogen with a long iron clip until the solution becomes solidified. Then, they remove the tubes and immediately irradiate them at 365 nm with a UV lamp. As in the above steps, students are asked to observe the differences between luminescence in ethanol and that in hexane at 77 K. The teacher then uses that model to explain the whole process.

Part 3: The Luminescence of 3-HF in Ordinary Filter Paper at RT and 77 \mbox{K}

The filter paper is cut into pieces 3 cm (long) \times 3 cm (wide). Similar to the steps in Part 2, the students place two filter papers in hexane and in ethanol (8.0×10^{-4} M), respectively, and then remove the papers from the cuvette to observe the luminescence at RT after the solvents evaporate. Subsequently, students immerse the papers in liquid nitrogen (77 K) with long iron tweezers for \sim 3 min, remove them from the cuvette, and immediately irradiate them at 365 nm with a UV lamp. They should note the rapid change in color because the temperature of the paper becomes higher in the air. If this step fails (i.e., no change is observed), the students can repeat the above steps to see the color change. Part 3 is an extended activity and raises questions for further discussion. The teachers can encourage students to offer their opinions or perspectives. The spectral results of 3-HF at different temperatures are also shown in Figures 2a and 4d to support the instructors' teaching (also see the Supporting Information).

HAZARDS

Safety goggles and gloves should be worn during the handling and storage of ethanol, hexane (nitrile gloves), and liquid nitrogen (cryogenic gloves). Liquid nitrogen can cause cold burns and should be handled with caution to avoid skin and eye contact. Small amounts of liquid nitrogen in liquid nitrogen dewars are recommended. When the glass tubes are immersed in the liquid nitrogen, they will freeze quickly and will become fragile. Students must be cautioned about not allowing them to collide with anything. If the activity in Part 3 is carried out, small amounts of hexane will evaporate to the air. Keep amounts low and keep bottles capped.

STUDENT RESULTS

Part 1: Building Molecular Models of 3-HF in Ethanol and in Hexane

Molecular models of 3-HF with ethanol and hexane are built, respectively, as shown in Figure 1a,b. In Figure 1a,b, a hydrogen



Figure 1. (a) 3-HF and ethanol and (b) 3-HF and hexane in the ground state. Colors of balls: white, hydrogen atoms; red, oxygen atoms; and black, carbon atoms. The positions of Velcro indicate the sites of hydrogen bonding.



Figure 2. Emission spectra and color of 3-HF (2 \times 10⁻⁵ M) in (a) ethanol and in (b) hexane at RT under irradiation at 365 nm.



Scheme 2. Schematic of 3-HF in Hexane and Its Tautomer

atom is covalently bonded to a highly electronegative atom, i.e., an oxygen atom, which attracts the electron density away from the hydrogen nucleus. This in turn allows the hydrogen nucleus Scheme 3. Schematic of 3-HF in Ethanol and Its Tautomer





Figure 3. Emission spectra and color of 3-HF (2×10^{-5} M) in (a) ethanol and in (b) hexane at 77 K under irradiation at 365 nm.



Figure 4. Emission spectra and color of 3-HF (8×10^{-4} M) in a filter paper immersed in ethanol (a and c), and in hexane (b and d) at RT and 77 K, and then removal of the papers from the cell to observe the luminescence under irradiation at 365 nm after the solvent evaporate.

to be simultaneously attracted to a lone pair of electrons on an electronegative atom in a neighboring group.⁵

Part 2: The Luminescence of 3-HF in Ethanol and in Hexane, at RT and 77 ${\rm K}$

The color appearance and luminescence spectra of 3-HF in ethanol and hexane at RT under irradiation at 365 nm are shown in Figure 2a,b. Although the intensity of luminescence in ethanol is weaker than that in hexane, the luminescence color in ethanol is similar to that in hexane. Comparisons are made with molecular models and drawings in Schemes 2 and 3. The blue

Scheme 4. Schematic Representation of 3-HF Absorbed on Filter Paper at RT in the Ground and Excited States



emission of the excited normal form cannot be observed in hexane. As can be seen in Scheme 2, in nonprotic solvent, i.e., in hexane, 3-HF will not interact with hexane through InterHB and instead forms the internally hydrogen-bonded complex (IntraHB) in the ground state, which can then tautomerize in the excited state. This behavior has been attributed to the very fast ESIPT process in 3-HF, which impedes F(Normal).9 This accounts for the green luminescence (Figure 2b). On the other hand, in protic solvent, i.e., in ethanol, 3-HF will exhibit IntraHB and InterHB with solute and solvent, respectively. Ground state equilibrium is established between internally and externally hydrogen bonded species.¹⁹ The favorable formation of H-bonded complexes between normal form and the protic solvent (InterHB) hence retards the ESIPT process and allows the observation of F(Normal), i.e., blue emission, in competition with F(Tautomer), i.e., green luminescence, which is from the ESIPT product (excited tautomer).⁵ Accordingly, dual luminescence from the internally and externally hydrogen-bonded solute can be observed (see Figure 2a and Scheme 3).²⁰ Note that from the time-resolved studies in polar protic solvent, it is shown that long components of emission decay of normal and tautomer forms differ, which indicate the absence of excited-state equilibrium.²¹ Furthermore, the reason why the green luminescence is the major part of the luminescence in ethanol is that from the excited normal state, a fast transition (\sim 50–100 fs) occurs to the excited tautomer state.²² The quantum yield of the excited normal form

and the excited tautomer form in ethanol is 0.008 and 0.016, respectively.²³ The intensity of the green luminescence, F(Tautomer), is 10 times that of F(normal, blue emission); the luminescence hence looks green.

For 3-HF at 77 K, the luminescence colors in the two solvents are completely different: blue in ethanol and green in hexane (see Figure 3a,b). This suggests that, in hexane, 3-HF molecules with IntraHB are in the ground state, and then ESIPT occurs upon excitation, giving rise to F(Tautomer). A similar result has also been reported by Bruker et al.²⁴ and McMorrow et al.²⁵ This phenomenon can be rationalized by the fact that hexane glass is "locally" soft, as in solid argon in 10K, and thus provides less resistance to ESIPT. Accordingly, the emission shows green tautomeric emission (major emission).²⁴ The violet luminescence in ethanol at 77 K is caused by poly-solvation of 3-HF by ethanol in the ground state, analogous to the 3-HF externally H-bonded solute/ solvent at RT. Poly-solvation denotes HB of solvent molecules to both the hydroxyl hydrogen and to the carbonyl oxygen on the central ring of 3-HF and this would effectively block IntraHB. However, at 77 K, the ground state equilibrium strongly favors poly-solvated InterHB species.²⁶ This InterHB form would then be "frozen-in" at 77 K, preventing the formation of the IntraHB species (the ground state precursor to the tautomer). On the other hand, the step from the excited state normal state to the excited tautomer state also requires a solvent-reorganization step, while it is inhibited in the frozen matrix at 77 K.²⁶ Thus, F(Normal) (major emission) can be observed.^{13,26,27}

Part 3: The Luminescence of 3-HF in a Filter Paper Immersed in Ethanol and in Hexane at RT and 77 K

In Part 3, 3-HF is further confined in filter paper immersed in ethanol and in hexane at RT and 77 K. The color appearance and luminescence spectra of 3-HF under irradiation at 365 nm are shown in Figure 4, a-d. The filter paper is immersed in ethanol and in hexane, respectively, and in both cases, the major emissions are green (Figure 4a,b). At 77 K, however, the filter papers show a blue emission (see Figure 4c,d). When students remove the filter paper from the glass sample cell, the solvent is allowed to completely evaporate off. The color change is known to result from the interaction between the filter paper, which is manufactured from cellulose, and 3-HF.²⁸ Similar to the concept in part 2, we can think of cellulose (with -OH group, see Scheme 4) as having a role similar to that of a protic solvent. In filter paper, the InterHB processes involving the cellulose in the paper are very similar to those that occur in ethanol, both at RT and at 77 K (also see Figures 2a and 3a). At RT, as 3-HF is adsorbed on the filter paper, 3-HF, with external H-bonding perturbation in the ground state, can undergo ESIPT, giving rise to the dual emission from the normal and tautomer forms (see Scheme 4 and vide supra). After the filter papers are soaked in liquid nitrogen (77 K) and removed, violet luminescence is observed. This is attributed to HB between 3-HF and cellulose in a confined space at this low temperature (cf. RT); i.e., the rate from the excited normal form is faster than that of the excited tautomer form, which mostly prevents the occurrence of ESIPT (vide supra).

CONCLUSION

Using luminescence in a naked-eye experiment conveys the differences in intramolecular (IntraHB) and intermolecular (InterHB) hydrogen bonding in the ground state and ESIPT in the excited state for students in general chemistry and physical chemistry courses. In addition, the students can construct IntraHB and InterHB of 3-HF in different solvents using the molecular models to better understand the concept and the interactions. This stimulates both interest in and discussion of the differences in luminescence at different temperatures. Last, in order to raise pedagogical creativity, the flexibility, the challenge, and interest in the activity, the luminescence of HB of 3-HF in ordinary filter paper is discussed as an essential component of the extensive study of the HB concept. In this hands-on activity, based on another viewpoint, we use luminescence in the excited state to "see" the HB in the ground state. Accordingly, this activity can be further extended to other molecular interactions.

ASSOCIATED CONTENT

Supporting Information

General information and materials, sample preparation, and results. This material is available via the Internet at http://pubs. acs.org.

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Notes

The authors declare no competing financial interest.

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