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Laboratories and Demonstrations

Effect of Solvents on the Fluorescence Emission Spectra of 1-Anilino-8-Naphthalene Sulfonic Acid: A Physical Chemistry Experiment

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This article describes an experiment that can be conducted to cover basic fluorescence concepts and study the effects of solvent polarity. It introduces students to fluorescence spectrophotometry and illustrates the effects of the solvent's dielectric constant and hydrogen bonding capability on fluorescence-emmision spectra.

Introduction

Fluorescence spectrophotometry is not always covered in spectroscopy classes at the undergraduate level. Although the polarity of the solvent is a very important factor in determining the physical properties of solutions, the effect of solvent polarity on fluorescence spectrophotometry is often not discussed in the physical chemistry laboratory. Fluorescence spectrophotometry can be included in the physical chemistry

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laboratory curriculum [1–4]. The following is an experiment that can be conducted to cover basic fluorescence concepts and study the effects of solvent polarity [5]. It is designed to introduce students to fluorescence spectrophotometry and also to teach them about the effect of the solvent's dielectric constant, hydrogen bonding, or both on fluorescence-emission spectra. Because the molar polarizability of a solvent is a fraction of it's dielectric constant (Mosotti–Clausius equation), both parameters are directly proportional. We will be using both terms (polarity and dielectric constant) interchangeably throughout this paper.

This experiment measures the fluorescence-emission spectra of the fluorophore 1-anilino-8-naphthalene sulfonic acid (1,8-ANS), Figure 1, in solvents of different polarities and different hydrogen bonding strength. The solvents used in this study are acetonitrile, ethylene glycol, butanol, ethanol, methanol, dimethyl sulfoxide, and pure water, but others such as THF are possible [5]. As a safety precaution we avoid the use of both DMSO and THF. Students can study the relationship between red or blue shifts in fluorescence-emission spectra, the dielectric constants of different solvents used, and the presence of hydrogen bonding. Generally changes in the quantum yield and a red shift in the fluorescence-emission spectra are observed as the dielectric constant of the solvent is varied.

Background

A vapor or a liquid irradiated by light is, under suitable experimental conditions, able to emit light. Light re-emission is called fluorescence if there is no change in electron spin in transition. When the exciting light is shut off, fluorescence usually decays in a very short time. Fluorescence can be observed from a monatomic vapor such as mercury or from atoms with a complex ground state such as thallium, lead, or antimony. In diatomic molecules the fluorescence phenomenon becomes more complicated, as there are more possibilities for absorption and emission. Pure liquids are capable of fluorescing; in fact, it was in liquids that the phenomenon of fluorescence was first discovered [6]. Some substances, when excited by absorbing light, can re-emit the light over longer periods of time (luminescence).

The best examples of fluorescence are those of organic substances in solution. Very complicated theories have been developed to explain the fluorescence in pure liquids and solutions. In general, substances which possess a complex chemical structure

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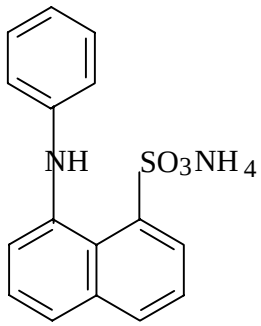

FIGURE 1. 1-ANILINO-8-NAPHTHALENE SULFONIC ACID, AMMONIUM SALT.

(molecules with a high molecular weight, many functional groups, and an extended π system) are the most likely to have a strong fluorescence emission. Considering that in complex molecules the quickest route to the ground state may be an electronic transition, one can expect more fluorescence from complex molecules. If one excludes the fluorescence of the solvent itself, then the effect of solvent polarity on the fluorescence of a solute can be measured.

The polarity of a solvent will generally influence the fluorescence-emission spectra of fluorophores. Changes in quantum yields and shifts in spectra are valuable parameters of fluorophore sensitivity to the solvent polarity. Quantum yield is the ratio of total number of emitted photons (number of emitted photons is represented by the area under a fluorescence emission-spectrum) to the total number of photons absorbed. The sensitivity of fluorophores to solvent polarity has practical applications in the field of physical biochemistry. When fluorophores are bound to proteins, nucleic acids, membranes, or macromolecules, in general, the fluorescence-emission spectra change. These changes can be employed to detect binding sites on macromolecules or to determine the polarity of binding sites. Changes in the emission spectra and a change in the quantum yield, or both may be observed [5].

Fluorescence spectra, under ordinary conditions of temperature and in condensed media, exhibit a red shift relative to the excitation radiation. A red shift , called a Stokes shift, is more common due to the efficiency of vibrational relaxation, which leads to a loss of energy before fluorescent emission. The loss of energy results from several dynamic processes, including dissipation of vibrational energy, reorientation of the solvent molecules around the excited state dipole, redistribution of electrons in the solvent molecules as a result of the altered dipole moment of the excited fluorophore,

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and fluorophore-solvent interactions (such as hydrogen bonding) [5]. Electronic excitation of aromatic compounds typically results in an increase in the dipole moment; thus, excited molecules exhibit larger dipole moments than molecules in the ground state. The increased dipole moment perturbs the environment surrounding the fluorophore. The solvent responds to this change by reorganizing its molecules around the fluorophore (solvent relaxation). The degree of reorganizing of the solvent molecules is dependent on the physical and chemical properties of the solvent and the fluorophore.

General solvent effects on fluorescence spectra are represented by the Lippert Equation. The Lippert equation is the most widely used expression among some other complex equations. In all of these equations the solvent is regarded as a network in which the fluorophore is embedded. The Lippert equation, although has its limitations, can be used to analyze experimental data with special considerations for general solvent effects [5].

Experimental Procedure

This experiment, including the preparation of solutions and conducting the spectrophotometric measurements, takes approximately three to four hours.

Materials, Chemicals and Equipment

The ammonium salt of 1-anilino-8-naphthalene sulfonic acid (1,8-ANS) can be purchased from ICN Pharmaceuticals (3300 Hyland Ave. Costa Mesa, CA 92626; Phone: 714-545-0100; www. icnpharm.com) or other biomedical supply companies. Spectral grade solvents with different polarities, such as acetonitrile, ethylene glycol, ethanol, methanol, butanol, dimethyl sulfoxide, and distilled water, are used. A fluorescence cuvette, eight 50-mL volumetric flasks, micropipettes, and eight 10-mL screw-top test tubes are needed as well as a UV–vis spectrophotometer and a fluorometer. The dielectric constants are obtained from a chemistry handbook [7], or (if desired) the student can measure the values needed provided a set-up such as a resonance apparatus is available [8].

Methods

Prepare 100 mL of a 0.005 M stock solution of the ANS ammonium salt by dissolving 0.1581 gram of the ANS ammonium salt (molar mass is 316.2 g mol^{-1}) in water. Take

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TABLE 1. Dilutions of ANS in different solvents used to obtain absorbencies of about 0.20 at a wavelength of 372 nm.

Name of solvent selected	Dielectric Constant	Volume of 1×10^{-4} M ANS solution (µL)	Volume of solvent added (mL)	Molar Concentration of solution ($\times 10^5$)
butanol	17.5	692	4.308	1.39
ethanol	24.55	701	4.299	1.40
methanol	32.70	704	4.296	1.41
acetonitrile	37.5	618	4.382	1.24
ethylene glycol	38.66	703	4.297	1.40
dimethyl sulfoxide	46.60	578	4.422	1.15
water	78.3	972	4.028	1.95

1-mL portions of the stock solution and mix each with one of the different seven preselected solvents in a 50-mL volumetric flask. These solutions are used to prepare a third set of solutions as indicated in Table 1. The same absorbance values for all the solutions are needed for the later comparison of fluorescence quantum yields and the absorbance value of about 0.20 or less is important for obtaining accurate measurements.

Students can use a UV–vis spectrophotometer to adjust the concentration of these solutions to obtain the same absorbance value at 372 nm [9]. Preparation of solutions with the same optical densities could be replaced by applying correction factors as shown later in the discussion section. The fluorescence-emission spectra of these solutions are then measured using a spectrofluorometer (a Fluoromax by SPEX was used in this case). A fluorescence-emission spectrum for each of the solutions is obtained by setting the excitation wavelength at 372 nm and collecting the emission fluorescence from 400 nm to 650 nm.

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Results and Discussion

If the spectrofluorometer used cannot provide an overlay of spectra on the same page, then the same plotting scale (as in the case of using an *xy* plotter) should be used. Inspection of Figures 2a and 2b allows observation of the shift in the fluorescence-emission spectra. Using these graphs and the refractive indices of the solvents also allows the relative quantum yield to be calculated for solvents of different polarities (see Table 2).

Absolute quantum yields are difficult to obtain experimentally and only very few absolute quantum yields have been determined. Absolute quantum yields can be calculated if the absolute quantum yield of a standard compound is available. For example, quinine bisulfate has a quantum yield of 0.546 at infinite dilution and 25° C at an absorption wavelength of 350 nm. Quinine bisulfate can be used as a reference to determine the quantum yield using an excitation wavelength of 350 nm [4].

The quantum yield, φ_1 and φ_2, of two solutions are related by [4]

$$\frac{\varphi_1}{\varphi_2} = \frac{\left(1-10^{-A_2}\right)n_1^2\alpha_1}{\left(1-10^{-A_1}\right)n_2^2\alpha_2} \qquad (1)$$

where A is the absorbance of the solution at the excitation wavelength, n is the refractive index, α is the area under the fluorescence-emission spectrum, and the subscripts refer to the solution number used in the calculations.

From the discussion above it is clear that quantitative calculations of absolute quantum yields can be obtained if a standard is used, but relative quantum yields can be obtained from equation 1. In equation 1, the expression involving the absorbance cancels if each of the solutions has the same absorbance. The area under the graphs of the spectra and the refractive indices can be used to obtain the quantum yield for each of the solutions relative to one of the solutions as a reference.

To obtain the ratio of the areas under the graphs, the graphs were plotted and the area of each was cut out using scissors and weighed. The ratio of the weights of the two pieces of paper, one for each graph, is the same as the ratio of the areas. This assumes a uniform thickness of paper so that the density of the paper cancels. The results are shown in Table 2. Fluorescence-emission spectra were obtained without a solvent

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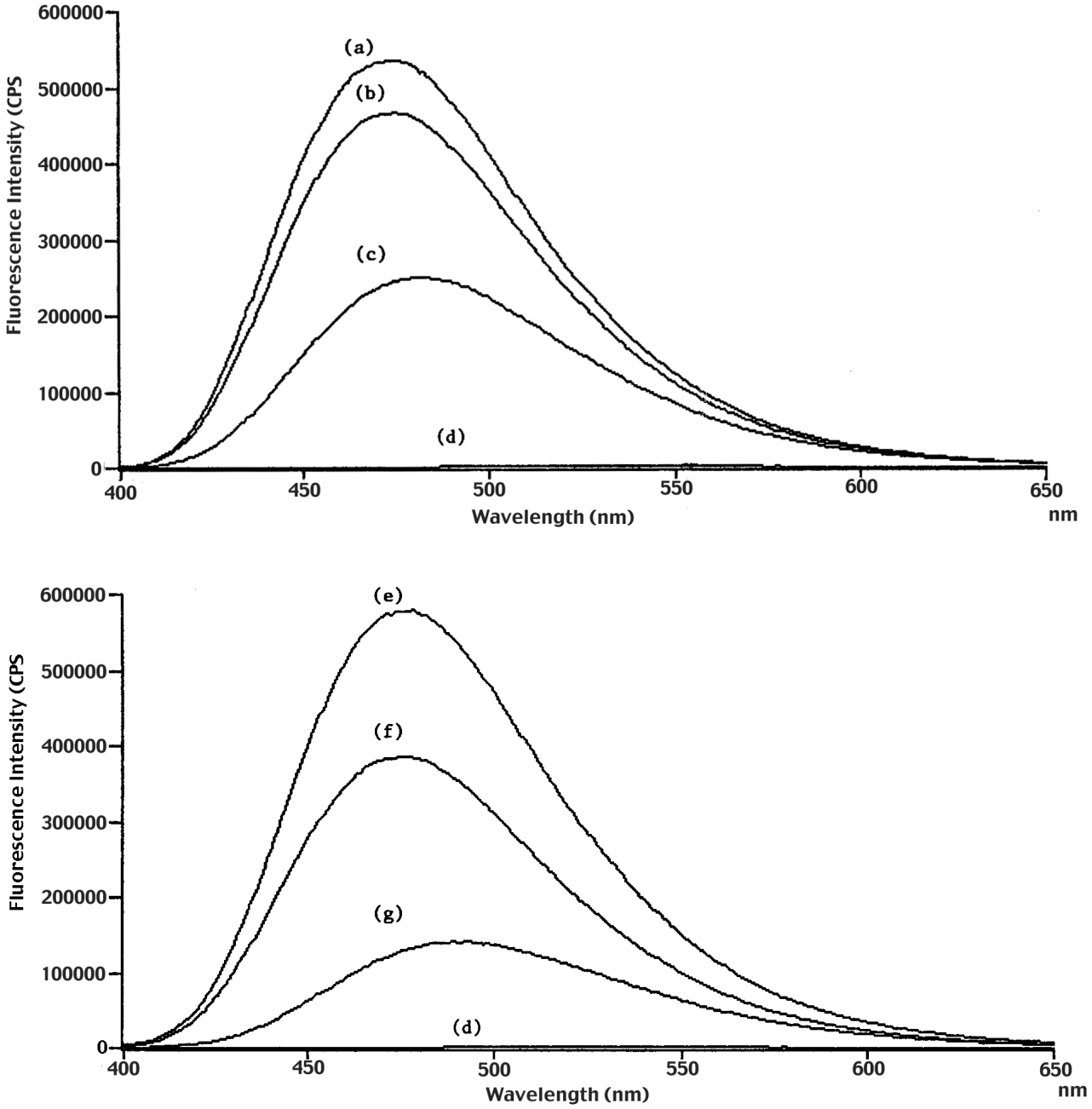

FIGURE 2. A) FLOURESCENE EMISSION SPECTRA OF ANS IN (a) BUTANOL, (b) ETHANOL, (c) METHANOL, AND (d) WATER. B) FLOURESCENE EMISSION SPECTRA OF ANS IN (d) WATER, (e) DMSO, (f) ACETONITRILE, AND (g) ETHYLENE GLYCOL.

baseline correction; however, emission spectra of solvents were obtained to guarantee the purity of solvents and it was determined that no solvent baseline correction is necessary. Spectra are, however, corrected for the instrument's detector response.

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TABLE 2. Solvent Refractive Indices, Wavelengths of Maximum Emission, and Relative Quantum Yields.

Solvent	Refractive Index, *n* [8]	Wavelength of Maximum Emission (nm)	Ratio of Square of Refractive Indices (n_1^2/n_2^2)	Ratio of Areas (α_1/α_2)	Relative Quantum Yields (φ_1/φ_2)
butanol	1.399	474.0[b]	0.896	0.91	0.815
ethanol	1,361	474.0[b]	0.848	0.82	0.695
methanol	1.328	483.0[a]	0.807	0.47	0.379
acetonitrile	1.344	476.0[c]	0.827	0.68	0.562
ethylene glycol	1.432	489.0[a]	0.939	0.31	0.291
dimethyl sulfoxide	1.478	478.0[c]	1.00	1.00	1.00
water	1.333	523.0[a]	0.813	0.018	0.0146

[a]Hydrogen bonding effect on wavelength of maximum emission.
[b]Weak hydrogen bonding.
[c]No hydrogen bonding.

In the application of equation 1, solution #2 has been chosen to be dimethyl sulfoxide while solution #1 is each of the solutions used with solvents other than dimethyl sulfoxide. This choice of reference makes all the values of the relative quantum yield less than unity.

The solvents in Table 2 can be divided into two groups, those which exhibit hydrogen bonding and those with little or no hydrogen bonding (see footnotes in Table 2). In all solvents the fluorescent emission of ANS is red shifted. The hydrogen-bonded solvents generally show a greater red shift than those which do not hydrogen bond. Butanol and ethanol seem to have a comparable emission maxima to that of acetonitrile. This is in agreement with the fact that alcohols with more than one carbon show weaker hydrogen bonding. Effect of the size of the alcohol molecule on the strength of hydrogen bonding is also obvious in methanol. Being the smallest alcohol, it exhibits a pronounced red shift in the wavelength of maximum emission, 483.0 nm. Water exhibits drastic changes in both wavelength shift and quantum yield, which can be

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attributed to the much greater hydrogen bonding present in water than in methanol. A comparison between acetonitrile and ethylene glycol, both with comparable dielectric constants, reveals the great effect of hydrogen bonding (as for ethylene glycol) on both the quantum yield and the wavelength of maximum emission for ANS. One can see that hydrogen bonding is the cause of decreased quantum yield and red shift in the spectrum and that the dielectric constant has minimal or no effect in this case.

The effect of hydrogen bonding on the quantum yield is observed in Table 2. As the amount of hydrogen bonding in a solvent is increased the quantum yield is decreased. In general one can conclude that larger quantum yields are observed in solvents with no hydrogen bonding. Although the dielectric constant is comparable in acetonitrile and ethylene glycol (Table 1) the quantum yield is twice as large in acetonitrile (Table 2).

Further discussion of solvent relaxation effects on fluorescence emission can be done by students based on a Jablonski diagram [5]. Students can be asked to elaborate on their observations and to provide a discussion of the process of fluorescence emission, the effects of excited state dipole moment, hydrogen bonding, and solvent relaxation on the number of emitted photons and on the emission wavelength.

Enrichment projects

1.The instructor may choose to have students determine the relative quantum yields. A separate laboratory period should be designated for that purpose in which students can determine fluorescence quantum yield using the methods of Stephen Bigger and his group [4].

2. Hydroxyl groups are known to exhibit stretching that could facilitate radiationless decay to the ground state. This would result in a subsequent drop in the quantum yield. In order to determine if this hypothesis is correct, students can compare the spectrum of ANS in H_2O with that in D_2O.

Conclusion

Students, as well as instructors, like this experiment because it is relatively short, requires minimum preparation of solutions, and generates a very small amount of chemical waste. Also, the results are consistent and reliable if care is taken in preparing solutions. In addition to using other solvents, other fluorophores, such as

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Phloxine B (a dye used in making pink-colored pistachios), 2-anilino-6-naphthalene sulfonic acid, 2-anilinonaphthalene, or 6-propionyl-2-(dimethylamino) naphthalene (PRODAN) can be used.

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REFERENCES

1. Shoemaker, D. P.; Garland, C. W.; Nibler, J. W. *Experiments in Physical Chemistry,* 5th ed.; McGraw–Hill: New York, 1989.

2. Sime, R. J. *Physical Chemistry*, 1st ed.; Saunders College Publishing: Philadelphia, 1990.

3. Schwenz, R W.; Moore, R. J. *Physical Chemistry*, 1st ed., American Chemical Society: Washington, DC, 1993.

4. Bigger, S. W.; Ghiggino, K. P.; Meilak, G. A.; and Verity, B. *J. Chem. Educ*. **1992**, *69*(*8*), 675.

5. Lakowicz, J. R. *Principles of Fluorescence Spectroscopy*, 3rd printing; Plenum Press: New York, 1986.

6. Hirschlatt, E. *Fluorescence and Phosphorescence*, 1st ed.;, Chemical Publishing Co.: New York, 1939.

7. Dean, J. A. Lange's Handbook of Chemistry, 13th ed.; McGraw–Hill: New York, 1985.

8. Chao, T. *J. Chem. Educ.* **1988**, *65*(*9*), 837.

9. Melhuish, W. H. *J. Phys. Chem*. **1961**, 65, 229.