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Original Communication

Utility of fluorophores as coatings for thin film based energy harvesting with micro-voltage driven smart sensors

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ABSTRACT

Many biological assays are used to measure fluorescence intensity in order to ascertain the number of fluorophore labeled probes that bind selectively to the molecule of interest in the assay. As the bound groups of molecules emit light upon excitation, fluorophores can be easily detected through various fluorescence spectroscopy techniques, including emission acquisition scans. This research involved utilizing the properties of these fluorophores and investigating their use in novel ways such as utilizing their energy yields to power microsensors and switches. Of the solutes studied, Quinine, Rhodamine B, Popop, and Anthracene, Rhodamine B yielded the highest fluorescent intensity (3.98×10^6 counts per second (cps)), when dissolved in ethanol, as well as the highest power measurement, 10.46 picoAmps (pA). Cyclohexane, methanol, sulfuric acid, and distilled water did not dissolve Rhodamine B as well as ethanol. Research within the laboratory continues to involve the analysis of fluorophore properties and activity in select solvents to discover effective and resolute solvents that may be used for other novel methods related to sensing and micro-based chemicals for energy harvesting.

KEYWORDS: smart sensors, micro-sensors, fluorescence spectroscopy, micro-voltage, energy harvesting, biosensors

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INTRODUCTION

Fluorescence spectroscopy is the study of luminescence, or the emission of light from any substance during electronic excitations. When in the electronically excited state and during electronic excitation, atoms are excited to high energy levels and can decay to lower (ground) levels through vibrational states providing an emission. The emitted photons will have different energies based on the materials properties, and therefore impart unique frequencies to the material being analyzed. Therefore, by analysing the different frequencies of light emitted in fluorescent spectroscopy, along with their relative intensities, the structure of the different vibrational levels can be determined [1]. Using fluorescence characteristics and its inherent short nanosecond decay, there may be a way to harness the energy from a fluorophore that has characteristics of a high level of energy, fluorescence with low light emission endurance, and strong photo stability upon exposure to various light levels within microsensors. For this study we investigated the properties of fluorophores in various solvents to obtain power measurements for their potential to power microsensors.

Within the past decade, microsensor devices have been powered by new technologies such as vibration harvesters typically that are linear mass-spring devices working at resonance [2], energy cells that scavenge light within carbon nanotube film (CNF) comprised of lead zirconate titanate cantilevers that are capable of converting light and thermal energies into electricity, which is based on triggering of CNF upon illumination by light

as well as thermal radiation and generation of an electric potential of 10 V [3], and organic light-emitting systems integrated with mechanical energy harvesting and energy storage polymer films that can be deformed and flexed to generate energy up to 0.5 mW within 100 s with ease [4]. Investigation of chemical properties of fluorescent based tagging materials when combined with novel thin films could yield improved energy harvesting apparatus within microsensing devices. This study focuses merely on the properties of the fluorescent tagging materials.

The majority of biological assays measure fluorescence intensity in order to ascertain the number of fluorophore labeled probes that bind selectively to the molecule of interest in the assay. Fluorescence radiance depends upon three parameters: the probability of absorbing a photon (molar extinction), the number of fluorophores, and the probability of radiative decay of the excited state (quantum yield) [1, 5]. As a group of molecules that emit light upon excitation, fluorophores are divided into two classes: intrinsic and extrinsic. Intrinsic fluorophores occur naturally, and include amino acids, NADH, flavins, and pyridoxal and chlorophyll derivatives. Extrinsic fluorophores are added to the sample to provide fluorescence, when none exists, or to change the spectral properties of the sample. Dansyl chloride, Fluoroscein, and Rhodamine B are considered extrinsic fluorophores because of these qualification properties [1]. Based upon the combination of the intrinsic and extrinsic properties in the majority of microsensors and provided the proper fluorescent characteristics are chosen then the harvesting of the energy from these combinations would yield higher power measurements. Therefore, these properties may allow for microsensors to have functionality.

There is a gap in optical research that involves an effective method to monitor characteristics of fluorophores dissolved in solvents over time that could provide efficient power to microsensor devices at the extrinsic level for energy harvesting applications within microsensing devices.

MATERIALS AND METHODS

The crystalloid and powdered forms of solutes $C_{14}H_{10}$ anthracene; $C_{24}H_{16}N_2O_2$ 1,4-Bis (5phenyloxazol-2-yl) benzene (Popop); $(C_{20}H_{24}N_2O_2)$ $2H_2SO_4$ $2H_2O$ quinine sulfate dehydrate; and $C_{28}H_{31}ClN_2O_3$ Rhodamine B were obtained from Sigma Aldrich and Fisher Scientific. Methanol, ethanol, cyclohexane, distilled water, and 1N standard solution sulfuric acid were utilized as solvents to determine the stability of each solution's fluorescence signature over the passage of a three-week period. Initially, 0.001 gram of each solute was added to 20 milliliter of each solvent shown in Figure 1 and diluted to an absorbance value of 0.1 on the Thermo Spectronic BioMate-3 Spectrophotometer shown in Figure 2. To monitor fluorophore activity, the samples were analyzed using a Jobin Yvon Horiba FluoroMax-3 luminescence spectrometer. The spectrometer recorded fluorescence intensity with excitation and emission scans with varying wavelengths and ranges, specific to each solute, as shown below in Table 1. The solutions, stored in twenty sterilized disposable scintillation vials, were situated in a dark laboratory refrigerator at 4 °C and were extracted for analytical purposes once a week for three weeks.

RESULTS

Power meter measurements were collected at each emission acquisition for each solute, shown in Table 1, and were recorded and categorized by the select solvent solutions over a three week period. The power measurements were recorded in picoAmps (pA), shown in Table 2. Table 3 shows the molar concentrations collected for each solution as well as the average molar concentration for each solute. Table 4 shows the solvent and absorbance values collected over week 1 and 3 for each fluorophore in solution.

DISCUSSION

A consistent trend occurred over the three-week time course. For example, emission acquisitions of anthracene at 350 nanometers (nm) excitation showed that the sulfuric acid poorly reflected the solute's capability to fluoresce with a value of 1.94×10^4 counts per second (cps) and a power measurement value of 1.8, 3.16, 2.85 pA. Cyclohexane and ethanol produced the highest emission acquisitions for anthracene at 1.97×10^6 (2.85 pA) and 1.97×10^6 cps (2.91 pA), as the solutions became fully saturated over time.

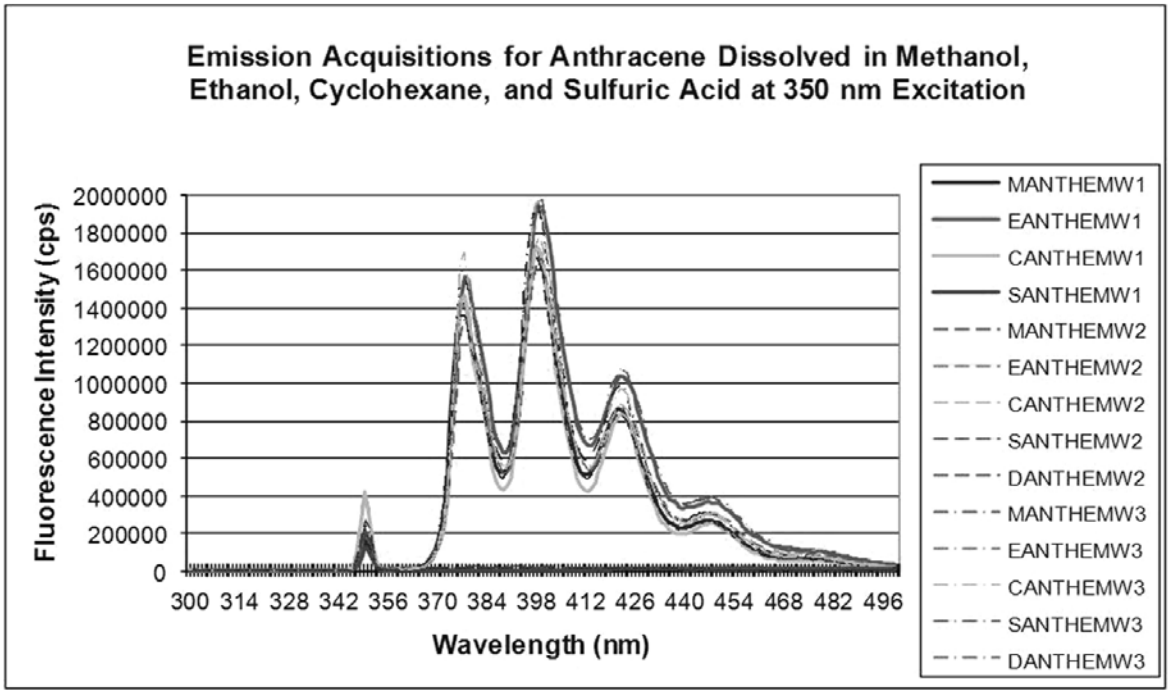

Figure 1. Emission acquisition for Anthracene. This figure is an emission acquisition from 300 to 500 nm for Anthracene dissolved in methanol, ethanol, cyclohexane, and sulfuric acid with an excitation fixed at 350 nm. Multiple measurements were obtained with the various molar concentrations and solutions. The fluorescence intensity was measured from 0 to 2.0 x 10^6 cps.

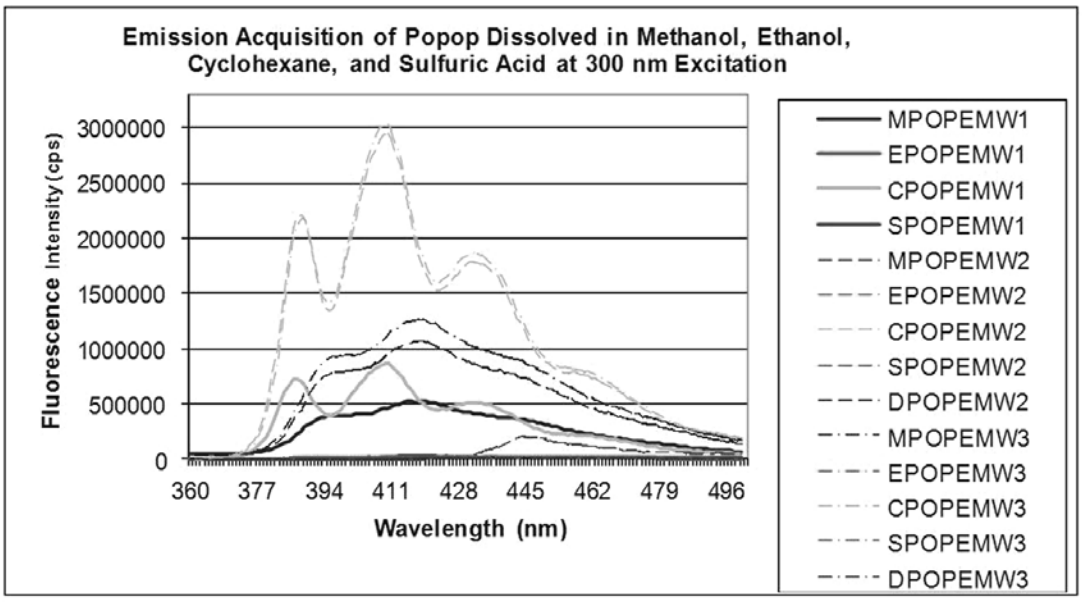

Figure 2. Emission acquisition for Popop. This figure is an emission acquisition from 360 to 500 nm for Popop dissolved in methanol, ethanol, cyclohexane, and sulfuric acid with an excitation fixed at 300 nm. Multiple measurements were obtained with the various molar concentrations and solutions. The fluorescence intensity was measured from 0 to 3.0 x 10^6 cps.

Table 1. Solutes and spectrometer parameters and ranges of measurement.

Solute	Emission acquisition (Ex)	Emission range	Excitation acquisition (Em)	Excitation range
Anthracene	350 nm	(300 - 500)	356 nm	(245 - 350)
Popop	300 nm	(360 - 500)	410 nm	(280 - 500)
Quinine	310 nm	(280 - 500)	450 nm	(280 - 500)
Rhodamine	550 nm	(500 - 750)	550 nm	(400 - 588)

Table 2. Solutes, solvents, and power meter measurements over a three week period.

		Power meter values (pA)		
		Week 1	**Week 2**	**Week 3**
Anthracene	Methanol	1.84	3.64	2.87
	Ethanol	2.95	3.11	2.91
	Cyclohexane	1.8	3.16	2.85
	Distilled water	1.62	3.03	2.28
	Sulfuric acid	3.06	2.66	2.34
Quinine	Methanol	2.35	3.09	2.98
	Ethanol	0.44	3.29	2.7
	Cyclohexane	0.01	2.97	1.75
	Distilled water	0.69	3.9	2.45
	Sulfuric acid	3.81	3.55	3.25
Rhodamine	Methanol	8.94	8.88	8.84
	Ethanol	10.46	9.25	9.03
	Cyclohexane	1.99	2.65	1.88
	Distilled water	4.23	6.2	6.02
	Sulfuric acid	5.07	5.16	4.99
Popop	Methanol	2.47	3.69	2.97
	Ethanol	1.16	3.1	2.3
	Cyclohexane	2.14	4.58	3.76
	Distilled water	1.39	3.21	2.16
	Sulfuric acid	3.05	2.6	2.21

Distilled water did not dissolve the anthracene, yielding little fluorescence (2.21×10^4 cps) and had a power meter value of 2.45 pA at the end of three weeks (shown in Figure 1). Emission acquisitions of Popop were similar to those of anthracene dissolved in distilled water.

Emission acquisition scans of Popop at 300 nm excitation, dissolved in varying solvents revealed that cyclohexane gave the highest yield, producing the highest fluorescence intensity as time progressed, while sulfuric acid faintly fluoresced, giving fluorescent intensity values of

Table 3. Molar concentrations for each solution, as well as average molar concentrations for each solute.

	Molar concentration
Anthracene	**3.19812E-04 M**
Sulfuric acid	3.08590E-04 M
Distilled water	3.08590E-04 M
Ethanol	3.08590E-04 M
Cyclohexane	3.08590E-04 M
Methanol	3.64700E-04 M
Quinine	**1.64760E-04 M**
Sulfuric acid	6.38600E-05 M
Distilled water	6.38600E-05 M
Ethanol	1.46880E-04 M
Cyclohexane	3.51230E-04 M
Methanol	1.97970E-04 M
Popop	**2.00336E-04 M**
Sulfuric acid	3.29320E-04 M
Distilled water	1.64660E-04 M
Ethanol	1.92100E-04 M
Cyclohexane	1.78380E-04 M
Methanol	1.37220E-04 M
Rhodamine B	**1.02294E-04 M**
Sulfuric acid	1.04380E-04 M
Distilled water	1.35700E-04 M
Ethanol	1.14820E-04 M
Cyclohexane	1.04380E-04 M
Methanol	5.21902E-05 M

3.03×10^6 and 2.02×10^5, respectively. This may be due to the prolonged exposure of cyclohexane in Popop, allowing full saturation of the solute within the solution. As the power meter readings indicate, the cyclohexane had a lower value (2.14 picoAmps (pA)) than the sulfuric acid (3.05 pA) at first, then slowly shifting to maintain a higher value (3.76 pA) by the third week of analysis (2.21 pA). Distilled water did not dissolve the Popop, and did not fluoresce with high intensity counts (1.11×10^4 cps) (shown in Figure 2).

Emission acquisition scans of Rhodamine B and ethanol showed the greatest fluorescence intensity (3.98×10^6 cps), and yielded the highest power meter readings for Rhodamine B upon each analysis: 10.46, 9.25, and 9.03 pA, respectively. Conversely, Rhodamine B showed little peak absorption (6.31×10^4 cps) upon an excitation of 550 nm when added to cyclohexane, also maintaining the lowest power meter values. Cyclohexane also quenched in Rhodamine B, resulting in a clear solution, contrasting to the

Table 4. Shows the solvent and absorbance values collected over week 1 and 3 for each fluorophore in solution.

Week	Solvent	Anthracene	Quinine	Rhodamine B	Popop
	Methanol	0.114	0.103	0.0005	0.001
	Ethanol	0.125	0.111	0.107	0.109
Week 1	Cyclohexane	0.0011	0.0055	0.0013	0.001
	Distilled water	0	0.001	0.104	0
	Sulfuric acid	0	0.11	0.001	0
	Methanol	0.067	0.098	0.092	0.246
	Ethanol	0.104	0.108	0.128	0.109
Week 3	Cyclohexane	0.106	0.01	0.244	0.998
	Distilled water	0	0.101	0.089	0
	Sulfuric acid	0.007	0.118	0.139	0.004

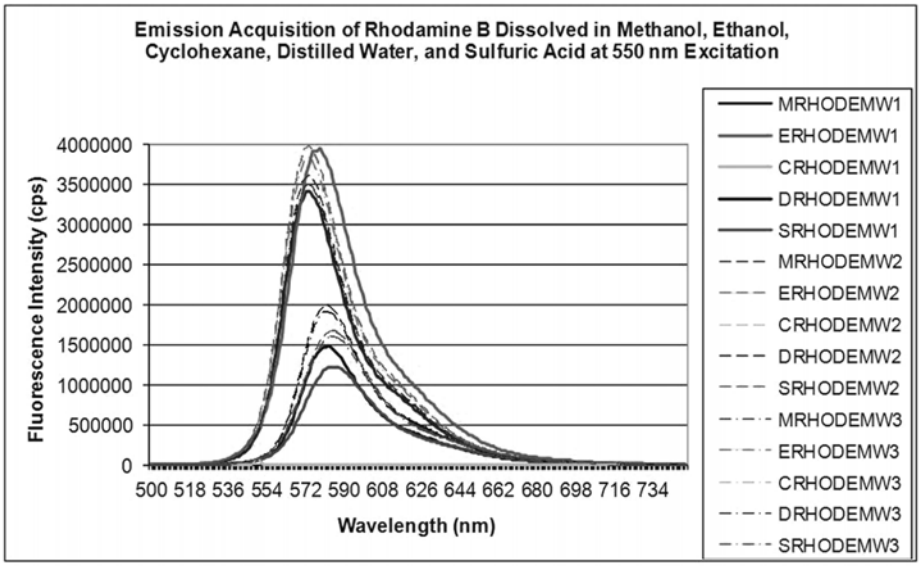

Figure 3. Emission acquisition for Rhodamine B. This figure is an emission acquisition from 500 to 750 nm for Rhodamine B dissolved in methanol, ethanol, cyclohexane, and sulfuric acid with an excitation fixed at 550 nm. Multiple measurements were obtained with the various molar concentrations and solutions. The fluorescence intensity was measured from 0 to 4.0 x 10^6 cps.

other brightly reddish-pink tinted solutions (shown in Figure 3). At a 450 nm excitation, a difference in emission acquisition peaks for quinine became evident with a red shift between each solvent. Cyclohexane did not dissolve the solute over the three-week period (shown in Figure 4), maintaining a peak intensity of 2.30 x 10^4 cps.

Upon refrigeration, all cyclohexane solutions froze, perhaps preserving and extending the life of each solution's fluorescence intensity. As crystalloid structures, anthracene and Popop did not dissolve as easily as powdered forms of Rhodamine B and quinine.

As shown in Figure 5, the largest power meter fluctuations occurred over the three week study period suggesting that Rhodamine B or other fluorophores yet to be studied that have resembling power yields would be the best candidates for

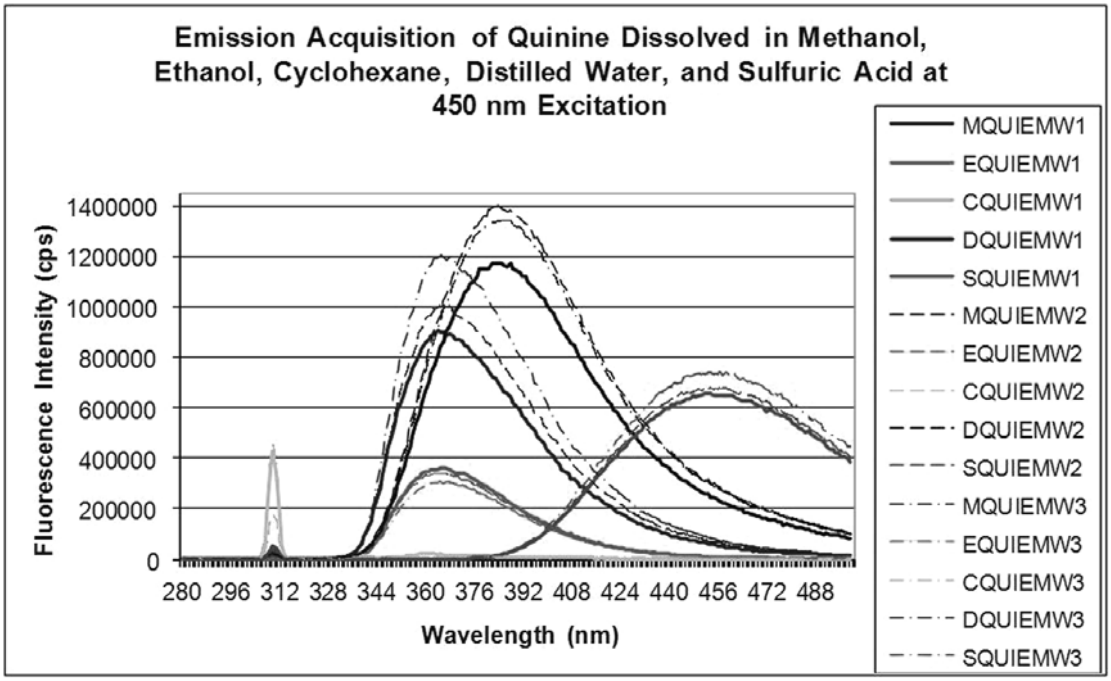

Figure 4. Emission acquisition for Quinine. This figure is an emission acquisition from 280 to 500 nm for Quinine dissolved in methanol, ethanol, cyclohexane, and sulfuric acid with an excitation fixed at 450 nm. Multiple measurements were obtained with the various molar concentrations and solutions. The fluorescence intensity was measured from 0 to 1.4 x 10^6 cps.

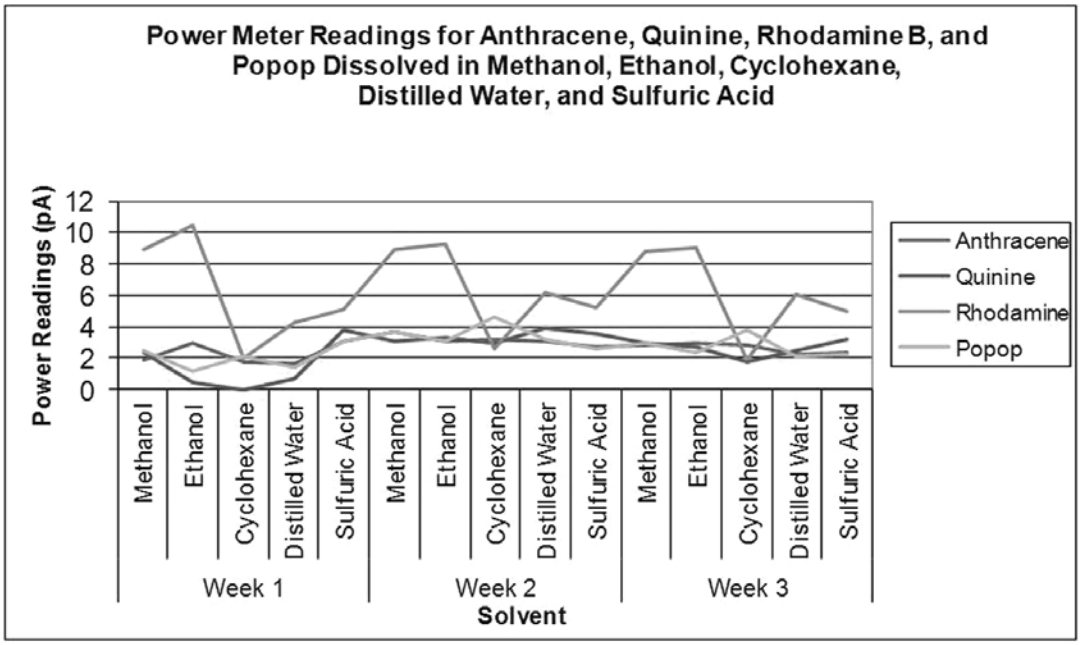

Figure 5. Power meter measurements. This figure is a chart showing the power meter readings for Anthracene, Quinine, Rhodamine B, and Popop dissolved in methanol, ethanol, cyclohexane, distilled water, and sulfuric acid. The chart shows the fluctuating power readings measured in pA over time.

energy harvesting at low energy levels with micro-sensors. This is also assuming that the micro-sensors could use these small energy bursts or outputs efficiently.

CONCLUSIONS

Extrinsic fluorescent energy harvesting techniques could be useful for monitoring fluorophore activity within aqueous solutions and providing new data to develop novel materials using fluorescent properties to yield energy to power microsensors. Most fluorescent materials are used to aid in marking non-fluorescent materials or biological samples for monitoring biological interactions and visualizing physiochemical characteristics [1, 6, 7, 8, 9]. By adding additional energy transfer characteristics to such a study could also aid in the enhancement of the energy optimization of the microsensor at the interaction of the intrinsic and extrinsic connection. An example of this is utilizing the properties of nanoparticle-based metal-enhancement, usually studied with silver, taking from immunoassay studies [10, 11]. As the enhancement provides an increase for the fluorescent emission or signature, it suggests there would be an energy increase as well. In addition, novel fabrication processes of polymeric piezoelectric films [12] may benefit with additional fiber coatings maximizing higher energy yields.

Sample measurements of the extrinsic fluorophores in this study indicated the inconsistent solubility of quinine, Popop, and anthracene in the various solvents. Quinine did not dissolve in cyclohexane, ethanol, or distilled water. In conjunction, Popop, a crystalloid, did not dissolve in ethanol and distilled water. Anthracene did not dissolve in distilled water. Rhodamine B yielded the highest fluorescent intensity (3.98×10^6 cps) of all of the solutes, when dissolved in ethanol, as well as the highest power measurement 10.46 pA. Laboratory research continues to analyze fluorophore activity to discover an effective and stanch solvent that may be used for other efficient methods of fluorescence remote sensing applications in micro- sensors and new energy harvesting applications. Future technology may one day be able to yield a usable functionality of such materials and solutions.

ACKNOWLEDGEMENTS

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CONFLICT OF INTEREST STATEMENT

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

REFERENCES

1. Lakowicz, J. R. 1999, Principles of Fluorescence Spectroscopy, 2nd Ed. Kluwer Academic/Plenum Publishers, New York.
2. Ferrari, M., Guizzetti, M., Ando, B., Baglio, S. and Trigona, C. 2010, Sensor Actuat A-Phys., 162(2), 425-431.
3. Kotipalli, V., Zhongcheng, G., Pathak, P., Tianhua, Z., Yuan, H., Yadav, S. and Long, Q. 2010, Appl. Phys. Lett., 97(12), 124102-3.
4. Chuo, Y., Landrock, C., Omrane, B., Aristizabal, J., Patel, J. N., Marzencki, M. and Kaminska, B. 2011, Sensors J., IEEE, 11(11), 2771-2779.
5. Masterton, W. L. and Hurley, C. N. 1993, Chemistry: Principles & Reactions 2nd Ed. Saunders College Publishing Fort Worth, Texas.
6. Dicesare, N. and Lakowicz, J. R. 2001, J. Phys. Chem., 105(28), 6834-6840.
7. Lakowicz, J., Shen, B., Gryczynski, Z., D'Auria, S. and Gryczynski, I. 2001, Biochem. Biophys. Res. Commun., 286(5), 875-879.
8. Anderson, J., Webb, S., Fischer, R., Kester, K. and Smith, C. 2002, Proc. of SPIE, 4576, 27-31.
9. Smith, C., Anderson, J. and Webb, S. 2002, Environ. Poll., 120, 517-520.
10. Geddes, C. D., Szmaciniski, H., Lakowicz, J. R. and Asian, K. 2004, J. Fluoresc., 14(6), 677-679.
11. Gupta, S., Huda, S., Kilpatrick, P. and Velev, O. 2007, Anal. Chem., 79, 3810-3820.
12. Yamashita, T., Takamatsu, S., Kobayashi, T. and Itoh, T. 2013, Transducers and Eurosensors XXVII, 17th International Congress, Barcelona June, 16-20, 1561-1564.