

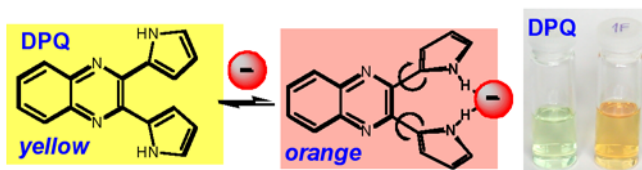
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We live in a chemical world. At all times we are surrounded by chemical entities, molecules, and ions. Most of the time we are not particularly aware of that, however, there are circumstances when being able to detect or visualize chemicals is of utmost importance. Sensing materials harmful to humans or animals or toxic to the environment necessitates development of sensors for chemicals, that is, chemosensors.<sup>1</sup> Sensor materials and devices allow us to translate the presence of a chemical entity to a clearly distinguishable signal output, such as change in color, electrical properties<sup>2</sup> or luminescence,<sup>3</sup> to name a few.<sup>4</sup> In our research at BGSU, we focus mainly on the aspect of optical sensing, using change in color<sup>5</sup> and luminescence to relay the information about presence of chemicals.

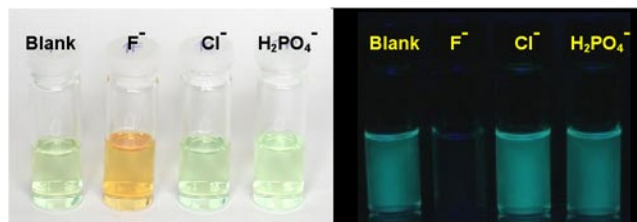
In this brief article we describe an example of developing optical sensors for inorganic anions. In fact, numerous molecules and materials capable of sensing anions while providing optical output have been reported, but materials that are inexpensive yet capable of working with anions administered in the form of purely aqueous solutions are still rare.<sup>6</sup> This is because compared to isoelectric cations, anions often display tautomerism, adopt a wide range of geometries, and possess low surface-charge density, features that make the binding and sensing of anions less effective.<sup>7</sup>

In this paper we show how we have converted 2,3-di(pyrrrole-2-yl)quinoxaline (DPQ) (Figure 1), a compound known in the literature since 1911<sup>8</sup> and only recently rediscovered as a potential colorimetric and fluorescent sensor<sup>9</sup> for anions, was converted into a viable sensor device. DPQ binds anions via concerted hydrogen bonding between the two pyrrole NH moieties and the anions. During the anion-binding process, changes in electronic density and conformation in DPQ result in a change in color as well as fluorescence (Figure 1).



**Figure 1.** The structures of 2,3-di(pyrrrole-2-yl)quinoxaline and DPQ-anion complex.

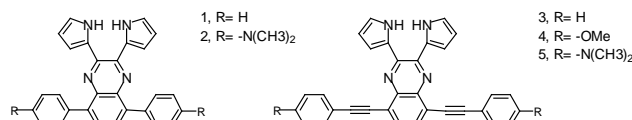
We have shown that the anion affinity and optical response of DPQ derivatives may be successfully tuned via attaching various substituents to the quinoxaline chromophore. In Figure 2, we show the anion-induced color and fluorescence in DPQ.



**Figure 2.** The changes in color and fluorescence of DPQ dichloromethane solutions upon addition of anions.

One can clearly see that the dichloromethane solutions of DPQ do change color, however, these changes are relatively weak. Also, the DPQ fluorescence is not very bright (quantum yield ~ 17%).

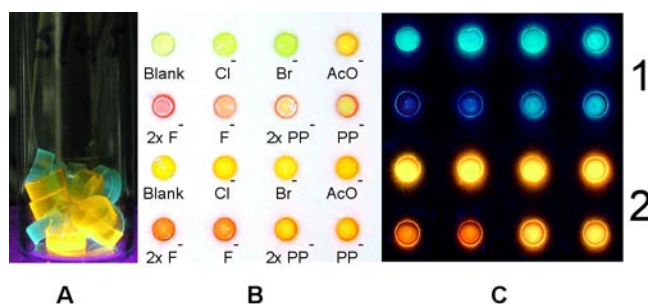
Because the fluorescence-based sensing is generally more sensitive, we decided first to improve the emissive properties of DPQ. This was achieved by attaching extended conjugated moieties to the quinoxaline chromophore either directly or via the acetylenic spacer, for example, in compounds in Figure 3.



**Figure 3.** The structures of DPQ-related sensors with extended conjugated chromophore.

While extending of the conjugated DPQ chromophore improved fluorescence properties by increasing the quantum yields from circa 15% to 25-35% depending on a specific substituent, the resulting materials required additional improvements to render them water-compatible so the anions could be administered as solutions in water or a buffer. This was achieved by embedding the DPQ-based sensors in hydrophilic polyurethane matrices. Medical-grade hydrophilic polyurethanes (Tecophilic™ by Thermedics™ Polymer Products, division of Noveon, Lubrizol) were custom synthesized using the company's proprietary technology. These polymer matrices were designed and synthesized in such a way that they take up a certain amount of water, bringing the soluble salts including an anion into close contact with DPQ sensors embedded within the polyurethane matrix.<sup>10</sup> In a way, the matrix extracts the anions from the bulk environment, together with a limited amount of water. The exact amount of water depends critically on the hydrophilicity of the polyurethane resin. The resulting sensor-doped

polyurethane was pressed into the shape of half-capsules of 20  $\mu\text{L}$  capacity (diameter = 4 mm, height = 2 mm) (Figure 4A). The capsules may be introduced in multi-well dishes used in automatic plate-readers or used in a variety of holders with machined wells. The aqueous analytes are then introduced into the sensor capsules and incubated for 30 minutes, after which the clearly observable color pattern appears. This process may also be visualized by black light (Figure 4). Also, the incubation process may be shortened to less than one minute by developing the assay in a common microwave oven. Figure 4B shows a 16-well Teflon plate in ambient light while Figure 4C shows the same 16-well plate illuminated by black light ( $\sim 365$  nm).

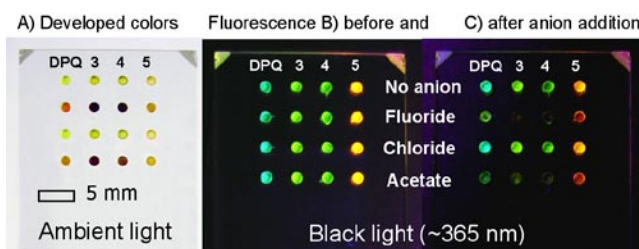


**Figure 4.** Panel A: Polyurethane capsules doped with sensors 1 and 2. Panel B: 16-well Teflon substrate with embedded capsules exposed to aqueous anion solutions. Panel C: The same 16-well assay illuminated by black light.

The multi-well capsule-based assay demonstrated numerous advantages of the polymer matrix-sensor approach, namely its flexibility in selecting the number and color/emission of the capsules to match the parameters of the detection instrumentation or, possibly, to circumvent colorant/fluorophore additives in food or beverages. However, the longer time required for swelling of the large capsule or required microwave heating to accelerate the process seemed somewhat impractical.

In the next stage, we have redeveloped this approach using micro-well plates. This new generation assays was fabricated by ultrasonic drilling of quartz slides to generate 500-750  $\mu\text{m}$  round holes 750  $\mu\text{m}$  deep (Figure 5). Polyurethane-doped sensors were solution cast to create about 100  $\mu\text{m}$  coating in the respective hole. The light emission may be observed by the naked eye or recorded using a fiber-optic detector. The addition of 0.25-0.5  $\mu\text{L}$  of an aqueous analyte solution to the micro-wells results in clearly observable color changes as well as changes in luminescence within seconds.

The rapid response of the material is attributed to its small size and a thin sensor film, which is not limited by analyte diffusion. The micro-assay is shown in Figure 5. The capacity of each well is circa 1  $\mu\text{L}$ , however, only 0.5  $\mu\text{L}$  is required to develop clearly observable color and emission patterns. The changes in fluorescence may easily be recorded using simple fiber-optic detectors coupled to hand-held computers for sample evaluation.



**Figure 5.** Microliter-scale assay. Sensors 3, 4 and 5 embedded in PU. Panel A: Clearly observable color patterns develop in the assay after anion addition. B: the micro-assay shows strong fluorescence from each micro-well. C: the addition of an analyte results in anion-induced change in fluorescence.

In summary, we show how the synergy in action between relatively ineffective colorimetric and fluorimetric sensors and polymer matrix results in a functional anion-sensor material and device. The beauty of this approach is that it does not require expensive synthesis, but rather relies upon simple mixing/matching protocols and is highly amenable to combinatorial optimization to meet various requirements of users and markets.

### Acknowledgment

This work was supported by NSF (DMR-0306117, NER-0304320, SENSORS grants), BGSU (TIE and FRC grants), and a Kraft Foods, Inc. grant. Financial support was also obtained from the McMaster Endowment.

### References

- (a) Schmidt, R. F.; Thews, G. *Human Physiology*, 2nd ed.; Springer-Verlag: Berlin, 1989. (b) Marshall, W. J.; Bangert, S. K. *Clinical Chemistry*, 5th ed.; Elsevier: Edinburg, 2004. (c) Ullmann's Encyclopedia of Industrial Chemistry, 6th Edition, Wiley VCH, New York, 1999.
- (a) Aldakov, D.; Anzenbacher, P., Jr. *J. Am. Chem. Soc.* **2004**, *126*, 4752. (b) Anzenbacher, P., Jr.; Palacios, M. A.; Jursíková, K.; Marquez, M. *Org. Lett.* **2005**, in press.

- (a) Anzenbacher P., Jr.; Tyson, D. S.; Jursíková, K.; Castellano, F. N. *J. Am. Chem. Soc.* **2002**, *124*, 6232.  
(b) Pohl, R.; Aldakov, D.; Kubát, P.; Jursíková, K.; Marquez, M.; Anzenbacher, P., Jr. *Chem. Commun.* **2004**, 1282.
- (a) Eggins, B. R. *Chemical Sensors and Biosensors*; John Wiley & Sons: Chichester, UK, 2002. (b) *Chemical and Biological Sensors for Environmental Monitoring*; Mulchandani, A., Sadik, O. A., Eds.; ACS Symposium Series 762; American Chemical Society, Oxford University Press, 2000.
- Nishiyabu, R.; Anzenbacher, P., Jr. *J. Am. Chem. Soc.* **2005**, *127*, 8270.
- (a) Martínez-Mañez, R.; Sancenón, F. *Chem. Rev.* **2003**, *103*, 4419. (b) Beer, P. D.; Gale, P. A. *Angew. Chem., Int. Ed.* **2001**, *40*, 486.
- Atwood, J. L.; Steed, J. W. In *Supramolecular Chemistry of Anions*; Bianchi, A., Bowman-James, K., Garcia-Espana, E., Eds.; Wiley-VCH: New York, 1997; pp 147-215.
- Oddo, B. *Gazz. Chim. Ital.* **1911**, *41*, 248.
- (a) Black, C. B.; Andrioletti, B.; Try, A. C.; Ruiperez, C.; Sessler, J. L. *J. Am. Chem. Soc.* **1999**, *121*, 10438.  
(b) Anzenbacher, P., Jr.; Try, A. C.; Miyaji, H.; Jursíková, K.; Lynch, V. M.; Marquez, M.; Sessler, J. L. *J. Am. Chem. Soc.* **2000**, *122*, 10268.
- Aldakov, D.; Palacios, M. A.; Anzenbacher, P., Jr., *Chem. Mater.* **2005**, in press.

### About the Authors

Manuel A. Palacios earned his B.S. in chemistry from the Simon Bolivar University in Caracas, Venezuela. He is a second year Ph.D. student in photochemical sciences at Bowling Green State University.

Pavel Anzenbacher, Jr. is an Assistant Professor of Chemistry at Bowling Green State University. A native of the Czech Republic, Anzenbacher was educated at the Charles University in Prague, where he received his M.S. degree from the Department of Organic Chemistry and earned a doctoral degree in organic chemistry from the Institute of Organic Chemistry and Biochemistry of the Czech Academy of Sciences in 1997. He served as a postdoctoral fellow at the University of Texas at Austin with Professor Jonathan L. Sessler. Anzenbacher's primary research interests include the development of advanced photonic materials in two main areas: supramolecular materials for molecular sensing and materials that can be used in the fabrication of flat displays. His e-mail address is pavel@bgsu.edu.

### Bowling Green Photochemists Receive Awards

Dr. Pavel Anzenbacher, (see article pp 18-20), received an Alfred P. Sloan Research Fellowship, a highly competitive national award designed to identify those who show the most promise of making fundamental contributions to the development of new knowledge in the sciences.

"Sloan Research Fellowships is the oldest program of the Sloan Foundation, though those who receive the grants are among the youngest researchers the foundation assists," according to the Sloan Foundation.

Fellows receive \$45,000 for a two-year period, to be used in setting up laboratories and establishing research projects. The Sloan funds give recipients the freedom to pursue whatever projects they feel are most compelling, and can be put to a wide variety of uses.

The foundation views the assistance of promising young scholars as an investment. "Financial assistance at this crucial point, even in modest amounts, often pays handsome dividends later to society," it states.

Anzenbacher has indeed shown promise. His research has already been recognized both by the University, with the presentation of the Outstanding Young Scholar Award in 2003, and externally, by his garnering of numerous support grants.

Dr. Felix Castellano was awarded the Olscamp Research Award which is given annually to a BGSU faculty member for outstanding scholarly or creative accomplishments during the previous three-year period. The quality of research is evaluated in terms of significance within the discipline, national and international import, artistic or scholarly creativity, and contribution to knowledge, culture or professional practice.

In the words of his nominator, Castellano has maintained an exemplary record over the past three years in a variety of research areas in his discipline of inorganic and organometallic photochemistry.

Since 2002, he has secured nearly \$1 million in extramural research grants and contracts. He is the recipient of a prestigious five-year National Science Foundation CAREER award and has received funding from both the Air Force Office of Scientific Research and the Department of Energy. He currently maintains 10 active collaborations and his work is known both nationally and internationally, with collaborators as far away as Italy, Ireland, the United Kingdom, the Czech Republic and France.