Chemiluminescence gets a boost from microwaves

Chemiluminescence-based methods of detecting biological processes at the cellular level work well because most samples do not generate unwanted luminescence that must be filtered out. Using these techniques, however, means that one is dependent upon the quantum efficiency of the chemical reaction and the depletion of the reactants over time.

Researchers at the University of Maryland Biotechnology Institute (UMBI) in Baltimore recently reported that placing samples on substrates immobilized with deposits of silver nanoparticles enhances chemiluminescence signatures. They reported that plasmons generated by chemiluminescence molecules in proximity to the surface boost the luminescence intensity of the sample particles. They named this effect "metal-enhanced chemiluminescence."

Now these researchers, led by Chris D. Geddes, professor and director of UMBI's Institute of Fluorescence, have added low-power microwave energy to this technique, reporting additional increases in luminescence intensity and faster reaction times. Furthermore, they developed the method using common materials that one can purchase at a discount retail store.

For chemiluminescent reactants, they used red, blue and green glow sticks — plastic tubes that contain a phenyloxalate ester, a fluorescent probe and a capsule containing hydrogen peroxide. Fluorescence occurs when the tube is bent, the capsule snapped and the chemicals shaken together. The investigators activated the glow sticks, placing ~10 µl of the fluid on silver island films — slides covered with silver dots that were 200 Å in diameter by 40 Å high. They also used unadorned glass coverslips as a control.

One hundred fifty seconds after luminescence initiation, they placed the slides inside a General Electric Co. consumer microwave oven (700 W of maximum power), set the power to 20 percent (140 W), and measured the spectra of the solutions before and after a single 10-s exposure of microwaves.

They decided on the 140-W setting because they determined it to be optimal. "The more power you put in, the faster the reaction," Geddes said. "Too fast, though, and you saturate the detector. Too slow, and you might as well use another technique." In addition, too high a setting could denature proteins, which they are investigating.

To obtain the spectra, the scientists used a spectrometer connected to a 1-mm-diameter fiber optic probe with a numerical aperture of 0.22 made by Ocean Optics Inc. of Dunedin, Fla. They used a consumer-model 3.2-megapixel digital camera made by Olympus Imaging America Inc. to acquire real-color photographs of the reagents.

According to Geddes, although the institute has tens of thousands of dollars' worth of equipment, they used a commonly available microwave cavity and digital camera because they wanted to create a simple and inexpensive system that would be easy to recreate.

They found that the microwave energy by itself enhanced the chemiluminescence of the reagents, whether glass or silver island films were used: from 22× for blue dye on glass slides to 85× for green dye on a silvered substrate. The differences in intensity among dyes on glass that were not irradiated and dyes on silver island film that were irradiated were more pronounced, ranging from 54× to 125×, depending on the dye color.

Microwaving also increased the reaction speeds, providing measurable responses in as little as 10 s, compared with up to 5 min for chemiluminescence reactions induced without microwaves.

The researchers are using the technique to analyze particular proteins — for example, ricin, a deadly toxin that must quickly be measured, Geddes said, down to the range of femtograms per milliliter of whole blood. □

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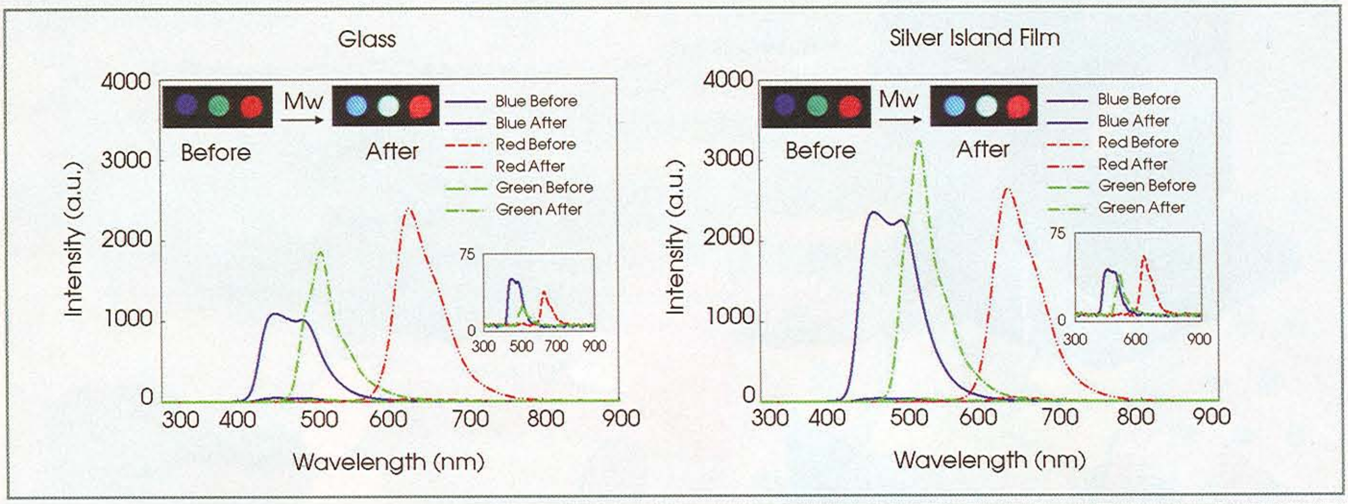

Researchers exposed chemiluminescent dyes to microwaves, thereby enhancing the luminescence intensity of the materials. Dyes were tested on either glass slides (left) or on silver island films (right), with those on the silvered substrates exhibiting the more pronounced enhancements. Mw = addition of 140 W of microwave energy; a.u. = arbitrary units. Reproduced with permission of the Journal of the American Chemical Society.