

Multicolor Microwave-Triggered Metal-Enhanced Chemiluminescence

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There is an increasing demand for the use of light-producing chemical reactions for quantitative detection in biotechnology, especially with regard to chemiluminescence-based ligand-binding assays.¹ The attractiveness of chemiluminescence as an analytical tool lies primarily in the simplicity of detection² since most samples have no unwanted background luminescence and no optical filters are required to separate the excitation wavelengths and scatter.² However, chemiluminescence-based detection is limited by the quantum efficiency of the chemiluminescence reaction or probe and the time before depletion of the reactants.² In this regard, an increased luminescence yield would clearly benefit overall detectability and therefore, for bioassays, the sensitivity toward a particular analyte.

Recently, the interactions of silver nanoparticles with chemiluminescent species, which resulted in an increase in the detectability of chemiluminescent reactions/species, with an approximately 20-fold increase in signal intensity, were reported by us and attributed to a plasmon-based luminescent enhancement.³ It was shown that surface plasmons can be directly excited by chemically induced electronically excited molecules. This phenomenon was named metal-enhanced chemiluminescence (MEC).³ In addition to their utility in increasing chemiluminescence intensity, silver nanoparticles, in combination with low power microwaves, were also shown to kinetically accelerate bioaffinity reactions for assays⁴ and immunoassays,⁵ which were monitored by fluorescence spectroscopy. The use of microwaves for the creation of chemically induced electronically excited states in a gas-phase reaction⁶ and in an ultrasound cavity⁷ has also been previously reported. However, the combined use of microwaves and metallic nanoparticles for the high sensitivity detection of chemiluminescence reactions has not *hitherto* been explored.

In this paper, *the proof-of-principle* of a new technique, which significantly enhances the luminescence intensity of chemiluminescence species and shortens the detection times by low power microwave heating in the presence of silver nanoparticles, called Microwave-Triggered Metal-Enhanced Chemiluminescence (MT-MEC), is presented. The applicability of the MT-MEC technique for multiple chemiluminescent species emitting at different wavelengths is also shown, which could be extended to any chemiluminescent detection system currently in use today.

The MT-MEC experiments were performed using blue, green, and red emission chemiluminescent reagents with and without microwave heating inside the microwave cavity on silver island films (SiFs) and on glass supports. For the proper comparison of the chemiluminescence (CL) data, the same initial CL emission intensity (150 s after initiating the CL reaction) for all the experiments was used.

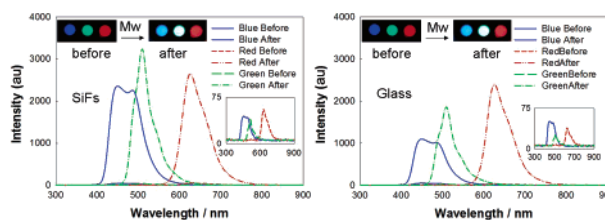


Figure 1. Chemiluminescence emission spectra (intensity: arbitrary units) of blue, green, and red chemiluminescent reagents (10 μ L) on silver island films (SiFs) (left) and on glass surfaces (right) before and after 10 s microwave (Mw) exposure. The insets show the spectra (before the Mw exposure) and the real-color photographs of the chemiluminescent reagents (before and after the Mw exposure).

Figure 1 shows the chemiluminescence emission spectra and the real-color photographs of the blue, green, and red chemiluminescent reagents on SiFs and glass surfaces before and after a single microwave exposure for 10 s (spectra were measured separately). The chemiluminescence intensity for all three reagents on SiFs and glass surfaces is *enhanced significantly* after the microwave heating, as also evidenced by the real-color photographs. The enhancement of CL intensity is due to two factors: (1) heating of the chemiluminescent species with microwaves in bulk (MT) distal from the metal, and (2) heating of the chemiluminescent species with microwaves close to silver nanoparticles (MT-MEC), where the enhancement is both due to the localized heating and due to the proximity of surface plasmons. Enhancement values related to these two factors are calculated⁸ and summarized in Table 1.

Table 1. Calculated Enhancement Values for CL Reagents Due to MT and MT-MEC

	blue	green	red
MT ₁ , SiFs: $I_{\text{SiFs}}(\text{Mw})/I_{\text{SiFs}}(\text{no Mw})$	54.0	85.0	69.0
MT ₂ , Glass: $I_{\text{glass}}(\text{Mw})/I_{\text{glass}}(\text{no Mw})$	22.0	75.0	63.0
MT-MEC: $I_{\text{SiFs}}(\text{Mw})/I_{\text{glass}}(\text{no Mw})$	54.0	125	70.0

After a single exposure to microwaves, the CL intensity due to MT was increased, ranging from 22- (blue, on glass) to 85-fold (green, on SiFs). The comparison of luminescence intensity on SiFs after the microwave exposure with the luminescence intensity on glass before the microwave exposure (i.e., MT-MEC) shows that the microwave heating of the chemiluminescent species in the presence of silver increases the CL intensity 54- (blue) to 125-fold (green) for a single microwave exposure.

To demonstrate the “on-demand” nature of the MT-MEC process, time-dependent chemiluminescent emission of the blue CL reagent on SiFs and glass surfaces, with multiple microwave exposures (Figure 2) and without any microwave exposures (Figure 2, inset), was recorded for 2000 s. The exposure of the blue CL reagent to microwaves (multiple exposures, all 20% power setting) results in

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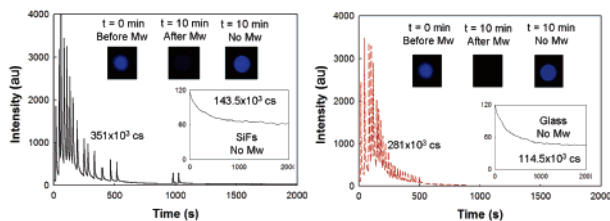


Figure 2. Time-dependent microwave-triggered chemiluminescence emission (intensity: arbitrary units) of blue chemiluminescent reagent ($10 \mu\text{L}$) on silver island films (SiFs) (left) and on glass surfaces (right) before, during, and after the Mw exposure. The initial intensities at $t = 0$ s for both graphs are 120 au. The “triggered spikes” in the intensity indicate the individual Mw exposure (10 s, 20% power). The inset shows the time-dependent emission (no Mw exposure) and the real-color photographs of the blue chemiluminescent reagent (before and after Mw exposure) on SiFs and glass surfaces. The area under each curve, that is, total photon flux, is given in terms of photon counts (cs). The final intensities at $t = 2000$ s for Figure 2 top and bottom are 30 and 25 au, respectively.

an increase in the CL emission, which is observed as “triggered spikes” consistent with the rising edge of the microwave pulses in the graph (microwave pulses not shown). The largest increase (120 to 3300 au on average) in CL intensity was observed during the first five microwave exposures and diminished upon further exposures, as chemiluminescent material is depleted. In all the experiments performed with low power microwaves, using both SiFs and glass, there was no evidence of surface drying. This is attributed to the previously made observations that the temperature increase of the aqueous solution on the surfaces due to microwave heating is only $\sim 8^\circ\text{C}$ (to $\sim 28^\circ\text{C}$) for a $30 \mu\text{L}$ aqueous sample.⁴

The number of photons detected from the blue CL reagent on the SiFs and glass surfaces after microwave exposures in 2000 s is 351×10^3 and 281×10^3 counts, respectively, which are significantly higher than those obtained *without* microwave exposures, 143.5×10^3 and 114.5×10^3 counts for SiFs and glass, respectively. This corresponds to a 2.45-fold increase in photon flux on both SiFs and glass surfaces (using SiFs yields 25% more flux than using glass) after microwave heating. These values also indicate that 50 and 70% of the total photon flux ($t = 0$ to ∞) of the blue CL reagent that luminesces for 4 h (Figure S1, Supporting Information) could be utilized within 10 min on demand, that is, triggered, on SiFs and glass surfaces, respectively, using microwave heating.

It is important to note that a 2.45-fold increase in photon flux represents the *average* increase in the overall photon flux from the ensemble of chemiluminescent species for 2000 s. One can complete the CL reaction with a single microwave exposure for 10 s that will yield similar photon flux for the CL reactions without microwave heating. Considering the fact that the CL reactions currently in use are usually completed within 5 min, MT-MEC technique provides researchers with an increased detectability and a significant reduction in CL detection time (to 10 s). In addition to the use of photon flux for CL reactions, one can also use CL intensity to obtain quantitative information for the CL reactions. In this regard, the use of SiFs (MEC) increases the CL intensity up to 2-fold (Figure 1, inset, and Figure S2) without microwave heating. On the other hand, the combination of low power microwaves with SiFs (MT-MEC) offers up to 125-fold increase (green) in CL intensity for a 10 s microwave exposure (Table 1).

The possible mechanism for the enhancement of the CL intensity by metallic nanoparticles has been described previously.⁹ It was

shown that the CL signature of luminol–hydrogen peroxide system is enhanced in the presence of gold nanoparticles. This was explained as originating from the catalysis of gold nanoparticles, which facilitates the generation of radicals and the electron transfer processes on the surface of the gold nanoparticles.⁹ In a separate study, it was shown that silver nanoparticles also enhance the luminescence intensity of CL species (MEC). MEC was hypothesized to be a distance-dependent phenomenon,⁴ where the enhancement of CL is thought occur due to the nonradiative energy transfer between the chemically induced electronically excited states of luminophores to the surface plasmons of the silver nanoparticles within 20 nm of each other. The contribution of microwave heating comes in to play with the *preferential heating* of the silver nanoparticles that result in the accelerated chemiluminescence reaction kinetics.

It should be noted that the *true* MEC enhancement factor here is much larger than 2. Considering the sample geometry used, where most of the CL species is in the bulk (the sample is 1 micron), then only 2% of the sample is within the MEC region (20 nm); hence the true enhancement factor is approximately 50 times larger (i.e., 100-fold MEC). This is in addition to 75-fold enhancement *solely* obtained by microwave heating. The true combined enhancement factor for MT-MEC is actually ~ 175 -fold. Subsequently, MT-MEC in essence provides a *unique platform technology* to rapidly collect (within 10 s) plasmon-enhanced chemiluminescence signatures. When employed to biodetection, MT-MEC will be a powerful alternative to Western Blotting for quantitative protein determination or even nucleotide detection. In this regard, work is underway in our laboratories and will be reported in due course.

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Supporting Information Available: The experimental conditions for the preparation of silver island films and the time-dependent chemiluminescence emission plots (without microwave heating) on SiFs and glass are provided. This material is available free of charge via the Internet at <http://pubs.acs.org>.

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- (8) MT is the intensity at 450, 510, and 625 nm after the Mw exposure divided by the luminescence intensity before the Mw exposure, for the blue, green, and red CL reagents, respectively. MT-MEC is calculated by dividing the luminescence intensity for SiFs after the Mw exposure by luminescence intensity for glass surfaces before the Mw exposure (MT-MEC: $I_{\text{SiFs}}(\text{Mw})/I_{\text{Glass}}(\text{no Mw})$).
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