Enhanced Performance from a Hybrid Quenchometric Deoxyribonucleic Acid (DNA) Silica Xerogel Gaseous Oxygen Sensing Platform

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A complex of salmon milt deoxyribonucleic acid (DNA) and the cationic surfactant cetyltrimethylammonium (CTMA) forms an organic-soluble biomaterial that can be readily incorporated within an organically modified silane-based xerogel. The photoluminescence (PL) intensity and excited-state luminescence lifetime of tris(4,7′-diphenyl-1,10′-phenanathroline) ruthenium(II) [$(Ru(dpp)_3]^{2+}$, a common O_2 responsive luminophore, increases in the presence of DNA-CTMA within the xerogel. The increase in the $[Ru(dpp)_3]^{2+}$excited-state lifetime in the presence of DNA-CTMA arises from DNA intercalation that attenuates one or more non-radiative processes, leading to an increase in the $[Ru(dpp)_3]^{2+}$ excited-state lifetime. Prospects for the use of these materials in an oxygen sensor are demonstrated.

Index Headings: **Deoxyribonucleic acid cetyltrimethylammonium; DNA-CTMA; Biomaterials; Oxygen sensor; Lifetime; Photoluminescence.**

Researchers have successfully implemented hybrid materials formed from deoxyribonucleic acid (DNA) and cetyltrimethylammonium (CTMA) in photonic and electronic applications. For example, DNA-CTMA has been used for electro-optic waveguide modulators,[1] organic light emitting diodes (OLED),[2] and organic field-effect transistors[3] and ultraviolet (UV) photodetectors.[4] Additionally, it is possible to create an interesting new range of optically active materials by adding luminophores to DNA-CTMA.[5–10] Due to the special architecture of DNA, different binding modes exists between DNA-CTMA and guest molecules: intercalation between base pairs and binding to the double helix minor or major grooves.[5] Also, DNA-CTMA has proven to be an attractive host material for non-linear optical dyes in comparison to conventional polymers such as poly(methyl methacrylate) (PMMA).[6] Here, DNA-CTMA thin films doped with sulforhodamine (SRh) exhibit a photoluminescence intensity more than an order of magnitude higher in comparison to SRh in PMMA.[7] Finally, many other fluorescence dyes have been reported to efficiently associate with DNA-CTMA.[6,8–10]

Some luminescent dyes are easily quenched by collisional quenching, ground-state complex formation (static quenching), or rearrangement of molecular chemical structure during the excited-state reaction.[11] Although such quenching is often considered a problem, one can exploit luminescence quenching to develop sensor platforms.[12–14] Oxygen is a well-known collisional quencher that can readily help to de-excite luminescent molecules.[15,16] As such, there have been numerous reports on the use of luminescence-based quenching for oxygen detection.[17–21]

Oxygen detection is particular important in biological, environmental, and industrial applications.[22,23] Luminophores like tris(4,7′-diphenyl-1,10′-phenanathroline) ruthenium(II), $[Ru(dpp)_3]^{2+}$, are commonly used for oxygen sensing. Based on prior research carried out in our laboratories using $[Ru(dpp)_3]^{2+}$,[24] and its longer fluorescence lifetime,[25] compared to $[Ru(bpy)_3]^{2+}$, we selected $[Ru(dpp)_3]^{2+}$ as the luminiphore in this research, rather than $[Ru(bpy)_3]^{2+}$ or other alternatives. In this paper, we explore the behavior of $[Ru(dpp)_3]^{2+}$ doped within organically modified class II xerogels that contain DNA-CTMA.

The following reagents were used: DNA (from marine salmon sperm); CTMA chloride; *n*-butanol (Sigma-Aldrich); $Ru(dpp)_3Cl_2{\bullet}5H_2O$ (GFS Chemicals); tetraethylor-

Received 18 December 2013; accepted 28 April 2014.
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DOI: 10.1366/13-07430

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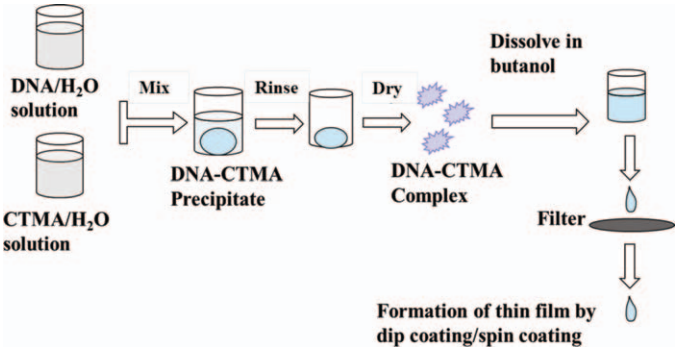

FIG. 1. Process procedures of DNA-CTMA complex.

thosilane (TEOS) (99.9%), and *n*-octyltriethoxysilane (C8-TriEOS; >95%; Gelest); HCl (ACS grade; J.T. Baker); and EtOH (200 proof; Decon Laboratories). All reagents were used as received. Deionized water was prepared by using an AmeriWater purification system (Metro Group) to a specific resistivity of at least 18 MΩ·cm.

The DNA material used in this research is typically a salmon fishing industry waste product. To obtain high optical quality thin films, the DNA is complexed with CTMA; this complex is soluble in polar organic solvents such as *n*-butanol. Figure 1 shows the process procedure of the DNA-CTMA complex. Molecular weight of the material can be decreased through sonication to suit application. An ultrasonic processor was used to decrease the molecular weight of the DNA so that it could be processed into an optical quality film. Molecular weight of DNA is a function of total sonication energy. The molecular weight is measured using agarose gel electrophoresis with a 0.8% agarose gel.[26] The DNA-CTMA sample used in this study is formed with a molecular weight of approximately 300 kDa.

The O_2 responsive xerogel used in this research was based on a formulation previously developed in our laboratories.[27] This particular xerogel was selected because it exhibits a good O_2–Volmer quenching constant (K_{SV}) and a linear Stern–Volmer response. Briefly, the all-silica sol was prepared by mixing, in order, TEOS (1.45 mL, 6.5 mmol), C8-TriEOS (2.05 mL, 6.5 mmol), EtOH (2.52 mL, 44 mmol), and HCl (0.800 mL of 0.1 M HCl, 0.08 mmol). The DNA-CTMA-doped sol was created by mixing 1 mL of the all-silica sol with 1 mL of DNA-CTMA (5% wt in *n*-butanol). Sols were capped and magnetically stirred under ambient conditions for 1 h. A luminophore-doped sol was prepared by mixing 20 μL of 25 mM $[Ru(dpp)_3]^{2+}$ (in EtOH) with 500 μL of the desired sol. A blank sol was prepared by omitting $[Ru(dpp)3]^{2+}$. Once the luminophore is added, these sols were capped and mixed for 2 min by a touch mixer and stored in the dark under ambient conditions for 24 h before use.[27]

The time-resolved intensity decay measurements were performed by using an N_2-pumped dye laser as the excitation source (Photon Technology International, model GL-301 dye and model GL-3300 pump). The dye laser output was adjusted to 448 nm. Xerogel film samples were formed by spin casting onto pre-cleaned glass microscope slides with spin speed 2000 rpm for 30 s. The sample emission was passed through a 570 nm long-pass fiber and detected with a photomultiplier tube

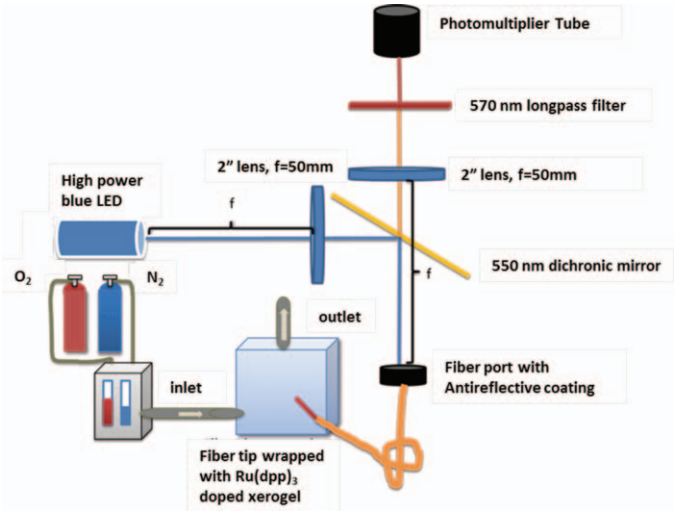

FIG. 2. Hybrid quenchometric DNA-silica xerogel sensing platform measurement setup. The xerogel sensor is coated on the fiber in the measurement chamber and the light emitted from the xerogel is carried by the fiber to the PMT.

(Hamamatsu, model R928). The photomultiplier tube output (terminated into 50 ohm) was connected to a 200 MHz digital oscilloscope (Tektronix, model TDS 350) that was interfaced to a personal computer. During these measurements, a pure gas or gas mixture was used to purge the entire sample chamber for 5 min and 10–20 data sets were collected when the total area under an intensity decay profile remained constant (2%). A CVI Lab Windows software program was used to acquire the data. The intensity decay profiles were analyzed by using Sigma Plot version 3.0 (Jandel Scientific). The short instrument response function (20 ns), combined with the long $[Ru(dpp)_3]^{2+}$ excited-state luminescence lifetime (>3 us), removes the need for deconvolution. All measurements were carried out at room temperature.

A low-cost and portable O_2 measurement system was built as shown in Fig. 2. A blue LED (λ_{peak} = 468 nm, Radio Shack, model 276-316) was used as the excitation source and driven by a function generator with 4.8 V, 2 KHz sinusoidal signal. A photomultiplier tube (PMT, Pacific, model 50B) was used to detect the emission photons with a 570 nm long-pass optical fiber. A lock-in amplifier (Stanford Research, SR830) was used to recover the emission signal and read-out using a computer.

O_2 and N_2 were mixed within a custom gas handling manifold with two separate inlets that are controlled by individual flow meters (Gilmont Instruments, GF 5542-1500). The xerogels were formed at the distal end of an optical fiber (Thorlabs, BFH37-200, 200 μm core, 0.37 NA) by removing a 2 mm segment of cladding and dip-coating the optical fiber into the aforementioned sols. The thickness of the film was 0.9 ~ 1.1 μm; it was characterized by profilometry with a surface profiler (Alpha Step IQ). The optical fiber serves to guide the excitation to the xerogel layer and deliver the emission signal to the detector. The proximal optical fiber end is connected to the optical fiber port using a SMA connector. The O_2 concentration surrounding the sensor

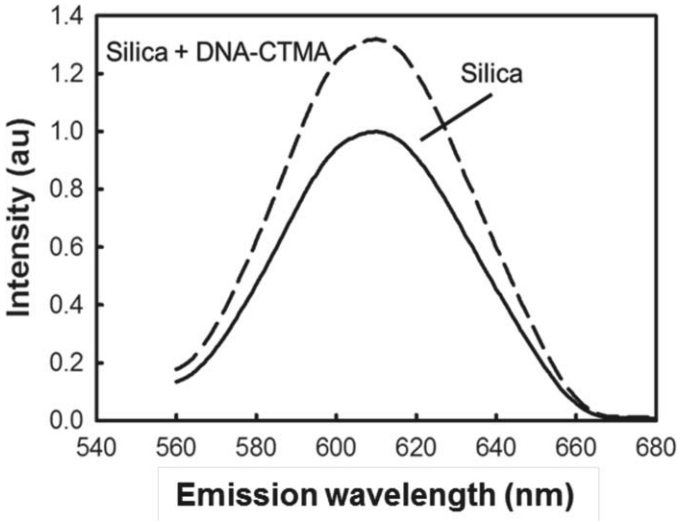

FIG. 3. Luminescence emission spectra for $[Ru(dpp)_3]^{2+}$ sequestered within silica-only and silica-DNA-CTMA xerogels.

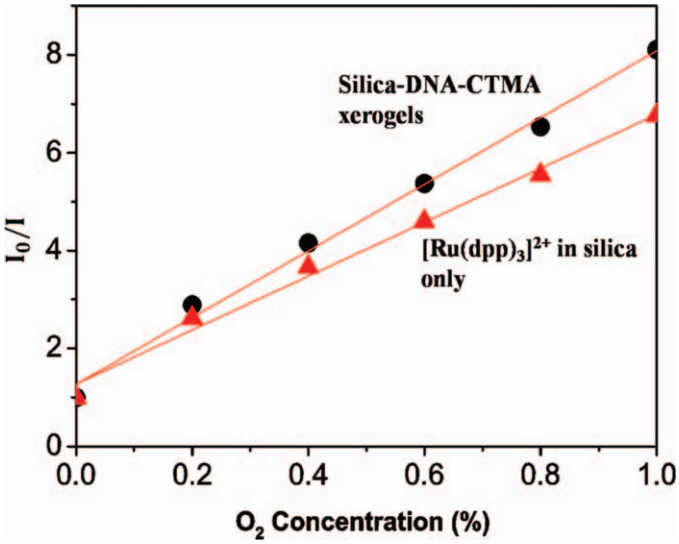

FIG. 4. O_2 Stern–Volmer plots for $[Ru(dpp)_3]^{2+}$ in silica-only and silica-DNA-CTMA xerogels.

was adjusted by changing its relative percentage with respect to N_2 concentration

Figure 3 presents steady-state luminescence emission spectra for $[Ru(dpp)_3]^{2+}$ sequestered within the silica-only and silica-DNA-CTMA xerogels in a 100% N_2 atmosphere. The $[Ru(dpp)_3]^{2+}$ emission maxima and full width at half maxima are unaffected by the DNA-CTMA; the emission intensity in the presence of DNA-CTMA is, however, increased by ~30%. Thus, the use of DNA-CTMA yields a sensor element that is 30% brighter in comparison to the xerogel without DNA-CTMA ($p = 10^{-4}$). How does the DNA-CTMA affect the $[Ru(dpp)_3]^{2+}$ O_2 response characteristics?

In the situation where a population of luminescent molecules are distributed within a matrix wherein all luminophores possess equal accessibilities to quenchers, the quenching process is described by the Stern–Volmer relationship[28,29]

$$I_0/I = 1 + k_q\tau_0[Q] \quad (1)$$

where I_0 is intrinsic luminescence without quencher; I is the luminescence in the presence of a quencher; k_q is bimolecular quenching constant between the luminophore and the quencher (depends on quencher diffusion, matrix transport properties, and the accessibility of the luminescent reporter to the quencher); τ_0 is the excited-state luminophore luminescence lifetime in the absence of quencher; and $[Q]$ is the quencher concentration. The term $k_q\tau_0$ is referred to as the Stern–Volmer constant (K_{SV}); in a quenchometric sensor, K_{SV} is the sensitivity.

Figure 4 presents O_2 Stern–Volmer plots for $[Ru(dpp)_3]^{2+}$ doped into silica-only and silica-DNA-CTMA xerogels. The Stern–Volmer plots are linear ($r^2 > 0.997$), demonstrating that the emitting $[Ru(dpp)_3]^{2+}$ molecules in the two xerogels are reporting from homogeneous microenvironments. This result is somewhat surprising given the complexity of the silica-DNA-CTMA xerogel matrix. Table I summarizes the O_2 response results for $[Ru(dpp)_3]^{2+}$ doped into silica-only and silica-DNA-CTMA xerogels. These data reveal that the K_{SV} value in the silica-DNA-CTMA xerogel is 20% greater in comparison to the silica-only xerogel ($p = 0.007$). Thus, the silica-DNA-CTMA material is brighter, and it exhibits a better O_2 response in comparison to the silica-only xerogel. The $[Ru(dpp)_3]^{2+}\tau_0$ for the silica-DNA-CTMA xerogel is 20% greater in comparison to the silica-only xerogel.

The recovered excited-state lifetimes and K_{SV} values are statistically different for each matrix. The k_q values are statistically equivalent, however. The underlying reason for the observed improvement in the response (K_{SV}) arises entirely from an increase in the $[Ru(dpp)_3]^{2+}$excited-state lifetime; the oxygen transport properties within the xerogel are unaffected by the DNA-CTMA. The increase in the $[Ru(dpp)_3]^{2+}$excited-state lifetime in the presence of DNA-CTMA likely arises from intercalation within the DNA matrix that attenuates one or more non-radiative relaxation processes leading to an increase in the $[Ru(dpp)_3]^{2+}$ excited-state lifetime. Surprisingly, the DNA-bound $[Ru(dpp)_3]^{2+}$ within the xerogel remains essentially as accessible to O_2 as free $[Ru(dpp)_3]^{2+}$within the same xerogel; DNA does not appear to impede O_2 access to the $[Ru(dpp)_3]^{2+}$.

A complex of DNA with CTMA is used to obtain hybrid DNA-doped xerogels. These new materials are brighter in comparison to the non-DNA-containing materials, and they also offer improved O_2 responses. The improvement arises entirely from an increase in the luminophore excited-state lifetime induced by the

TABLE I. Summary of photoluminescence lifetime, Stern–Volmer K_{SV}, quenching constant k_q of xerogel and xerogel + DNA-CTMA in nitrogen atmosphere.

Matrix	Lifetime (us)[a]	K_{SV} $(\%O_2)^{-1}$	k_q $((\%O_2)^{-1}s^{-1})$
Xerogel	4.8 ± 0.1 us	0.059 ± 0.002	$(1.2 \pm 0.2) \times 10^4$
Xerogel + DNA-CTMA	5.7 ± 0.4 us	0.072 ± 0.002	$(1.3 \pm 0.2) \times 10^4$

[a] Results were assessed for statistical significance by using analysis of variance at the 95% confidence level with pairwise comparison (Holm-Sidak test) ($p < 0.05$ being significant). In all cases, the power of performance test exceeded 0.97.

presence of DNA. The luminophore O_2 accessibility is unaffected by DNA.

ACKNOWLEDGMENTS

This research is supported by a grant from BerrieHill Research Corporation (BRC) under a program funded by the Air Force Laboratory at the Wright Patterson Air Force Base and the National Science Foundation under Grant No. CHE-0848171. Any opinions, findings, and conclusions or recommendations expressed in this material are those of the authors and do not necessarily reflect the views of the Wright Patterson Air Force Base and National Science Foundation.

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