Optical Sensing Material for Dissolved Oxygen: Covalent Immobilization of *Tris*(4,7-diphenyl-1,10-phenanthroline)ruthenium(II) Complex in Sol-Gels

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In recent years, much effort has been devoted to the development of optical sensors for dissolved oxygen (DO), because dissolved oxygen measurements in an aqueous environment are widely used in the biological,[1] environmental,[2] and industrial[3] areas.

The most common types of optical oxygen sensing materials are based on the dynamic quenching of the luminescence of dye molecules immobilized within a solid substrate. Therefore, the performance of the optical sensors strongly relies on the ability of the solid substrate to immobilize the oxygen-sensitive luminophore. To date, various solid substrates have been explored, including cellulose acetate,[4] fluoropolymers,[5] ion-exchange polymers,[6] poly(methyl methacrylate),[7] poly(styrene),[8] poly(vinyl chloride),[9] silicone rubber,[10,13] and sol-gel matrices.[11] Among these materials, sol-gel matrices are increasingly utilized to develop sensing materials, due to their superior chemical stability, optical transparency and porosity.[12]

Ruthenium(II) polypyridyl complexes are attractive fluorophores for use in fluorescence-based oxygen sensors owing to their high photochemical stability, high molar absorptivity, long lifetime derived from the metal to ligand charge transfer (MLCT) excited states, and large Stokes shift.[13-16] The immobilization of such Ru(II) complexes on sol-gel matrices has been recently investigated for the development of optical sensors.[17-21] Although most of these investigations involved the encapsulation (including impregnation and doping) of dye molecules into sol-gels, these methods could cause leaching of small dye molecules from the host sol-gels into the analyte solution during liquid-phase sensing, thereby reducing the sensor lifetime. To circumvent this leaching problem, an alternative method can be used involving the covalent binding of the dye molecules on the sol-gels. Therefore, in order to develop oxygen-sensing materials having long-term stability without any leaching problems, we explored the covalent immobilization of fluorophores in sol-gels. Herein, we report the preparation of an optical sensing material based on the covalent immobilization of a Ru(II) complex on a porous sol-gel matrix.

The *tris*(4,7-diphenyl-1,10-phenanthroline)ruthenium(II) complex [Ru(dpp)$_3$] (**1**, Fig. 1) is the fluorophore of choice in this work, since it is a well-known fluorescent dye which is quenched dynamically by molecular oxygen.[18] To our knowledge, the covalent immobilization of Ru(II) complex **1** in sol-gels has not previously been reported.

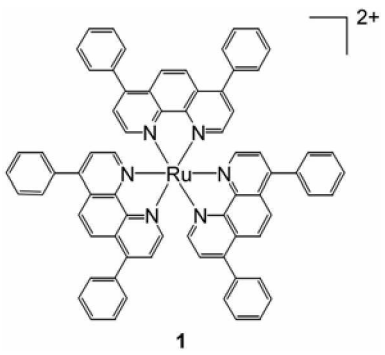

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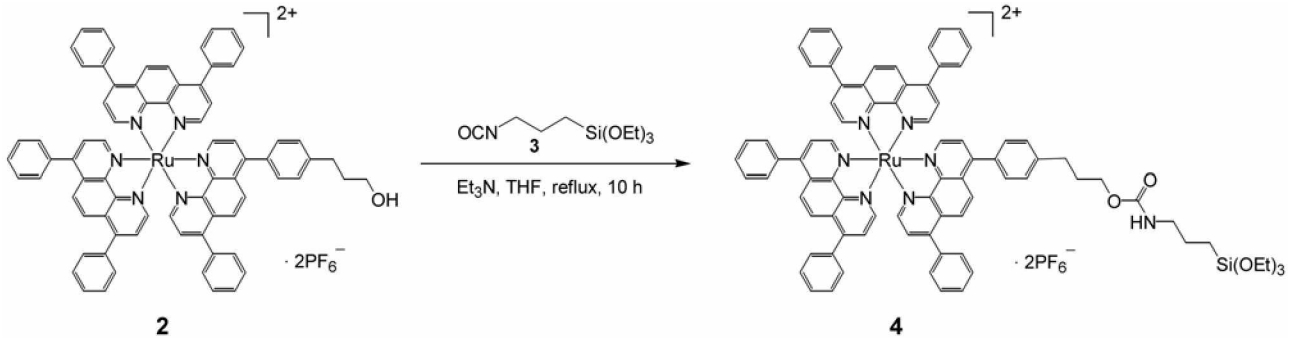
Figure 1. Tris(4,7-diphenyl)-1,10-phenanthroline)ruthenium(II) complex [Ru(dpp)$_3$].

In our previous work,[22] we described the synthesis of the *tris*(4,7-diphenyl-1,10-phenanthroline)ruthenium(II) complex (**2**) possessing a hydroxypropyl group, which is able to react with reactive silicate precursors such as 3-(triethoxysilyl)propyl isocyanate, 3-(glycidoxy)triethoxysilane, or 3-chloropropyltriethoxysilane. With this complex **2** in hand, we initially attempted to prepare a sol-gel precursor, **4**, linked with Ru(dpp)$_3$, which can be readily bound to sol-gels. Thus, the chemical bonding of complex **1** to a silicate precursor was achieved by the reaction of complex **2** with a slight excess (1.2 eq.) of 3-(triethoxysilyl)propyl isocyanate (**3**) in boiling tetrahydrofuran in the presence of triethylamine as a catalyst under a nitrogen atmosphere to afford the precursor **4** in good yield (Scheme 1). A small amount of unreacted complex **2** was observed in thin layer chromatography on silica gel eluting with a 20:1:1 mixture of CHCN/saturated aqueous KNO_3/H_2O. The R_f values are 0.60 for the complex **2** and 0.55 for the silicate derivative **4**, respectively. The coupling between the two components was confirmed by the formation of a carbamate moiety (–$NHCO_2$–), which was characterized by the corresponding stretching vibrations (FT-IR) both at 3300 cm^{-1} (N-H) and 1700 cm^{-1} (C=O). Further structural confirmation of **4** was supported by the molecular peak (MALDI-TOF MS) at 1403.49 for $[M\text{-}2PF_6]^{2-}$.

The covalent immobilization of Ru(dpp)$_3$ in the sol-gel matrix (M1) was accomplished by the reaction of the silicate derivative **4** with a sol-gel solution containing tetraethyl-



Scheme 1

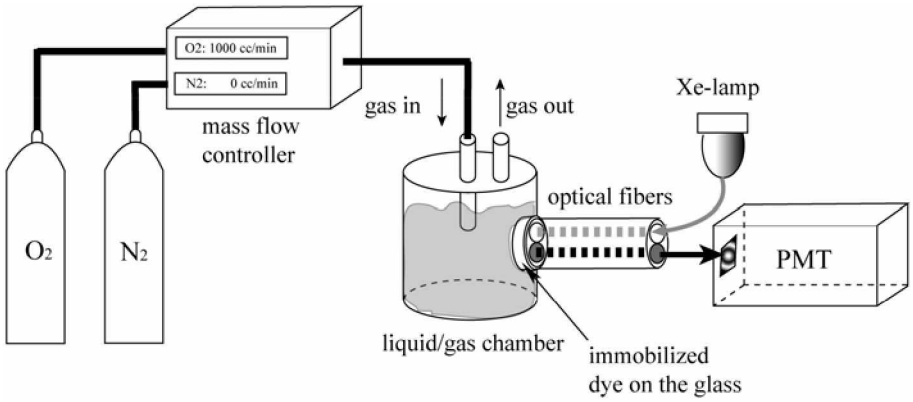


Figure 2. Schematic of experimental system used to measure the fluorescence intensity of the sensing membranes.

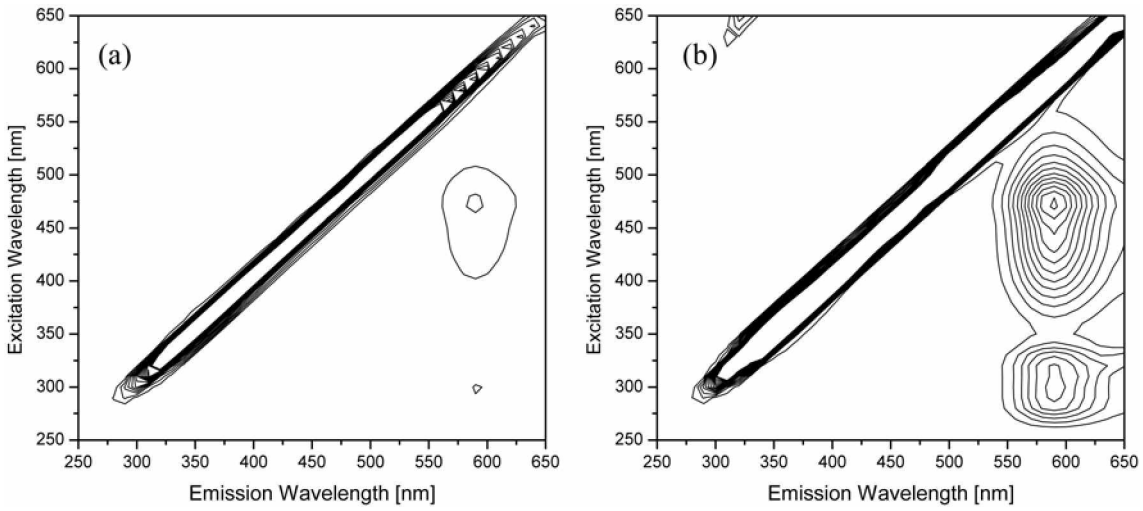


Figure 3. 2D fluorescence spectra of the sensing membrane M1 in aqueous solution saturated with O_2 (a) and N_2 (b).

orthosilicate (TEOS) under acidic conditions. TEOS is one of the most widely used sol-gel precursors for immobilizing optical sensors in sol-gels.[12] The sensing membrane was obtained by casting a fixed volume of the sol-gels onto a microscopic slide with a manual pipetting technique. For comparison purposes, the doped sol-gel membrane (M2) was also prepared from the mixture of $Ru(dpp)_3$ (**1**) and the same sol-gel solution.

The 2D fluorescence spectra of the covalently immobilized sensing membrane (M1) are shown in Figure 3. Like $Ru(dpp)_3$,[21] this membrane exhibits a strong fluorescence emission at 590 nm when excited by radiation of 470 nm in aqueous media de-aerated by bubbling N_2 gas. As expected, the fluorescence emission intensity was reduced by the quenching process when the membrane was exposed to an aqueous solution saturated with O_2 gas. Figure 4 shows the typical quenching response of the sensing membrane, M1, towards the same concentration of dissolved O_2. This test was performed by continuously exposing the sensing membrane to a mixture of dissolved O_2 and N_2 obtained by bubbling the two gases. The membrane, M1, is regenerated by flushing with N_2 gas, and there was no drift in any of the

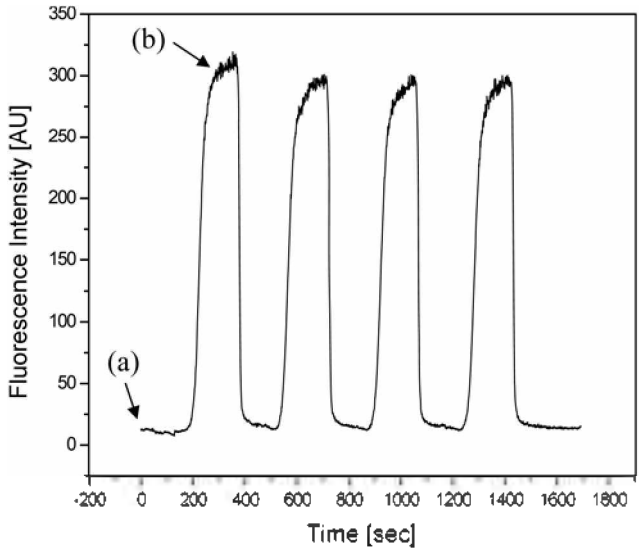


Figure 4. Quenching response of the sensing membrane M1 to dissolved oxygen when the solution is saturated with O_2 (a) and N_2 (b). Excitation at 470 nm, fluorescence observed at 590 nm.

signal changes, indicating that membrane M1 can therefore be used for repeated measurements.

To compare the extent of dye leaching in order to evaluate their long-term operation, the sensing membranes, M1 and M2, were soaked in distilled water separately. Periodically, the membranes were removed from the water and their fluorescence intensities were measured at 590 nm with an excitation wavelength of 470 nm. As depicted in Figure 5, in the case of membrane M2, $Ru(dpp)_3$ leached out of the membrane and only about 40% of the $Ru(dpp)_3$ originally present in the gels was retained after 40 days. In contrast, the $Ru(dpp)_3$ in membrane M1 did not leach during the same time period. These results demonstrate that their covalent immobilization in gels prevents the dye molecules from leaching out of the matrix, although a slight decrease of the emission intensity for membrane M1 was observed during

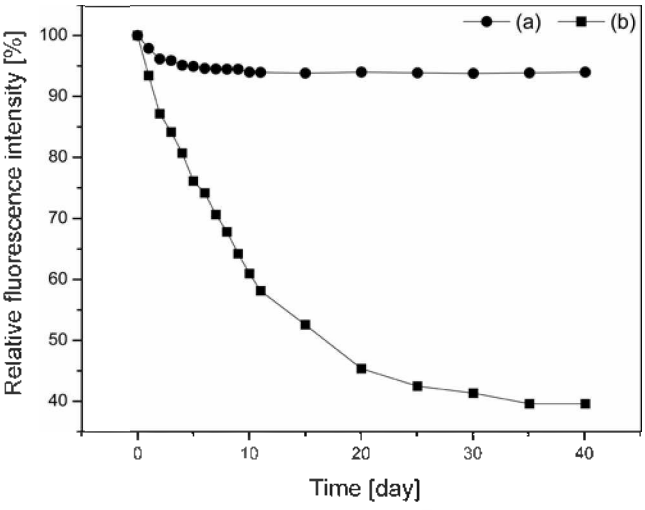


Figure 5. Leaching of the dye molecules from the membranes M1 (a) and M2 (b) in water.

the initial stage of the experiment. This decrease in intensity is probably due to the leaching of the unreacted complex **2** within the gels. In addition, we found no changes in the fluorescence intensity when membrane M1 was kept in air for three months.

In conclusion, the silicate derivative of the fluorophore, $Ru(dpp)_3$, was synthesized and firmly bound to a TEOS-based sol-gel matrix through covalent bonding to provide a suitable optical sensing membrane for dissolved oxygen measurements. This membrane showed long-term stability and repeatability. The leaching of the dye was prevented by its covalent immobilization in a sol-gel matrix.

Experimental Section

IR spectra were recorded on a Shimadzu FTIR-8300 spectrophotometer. MALDI-TOF mass spectral data were obtained on a Voyager DE-STR proteomics analyzer. The optical properties were measured with a Hitachi F-4500 fluorescence spectrophotometer. All reagents were obtained from Aldrich Chemical Co. and used without further purification. THF was dried by refluxing with benzophenone/Na under an N_2 atmosphere. Complex **2** was prepared according to the reported procedure.[22]

Preparation of sol-gel precursor linked with *tris*(4,7-diphenyl-1,10-phenanthroline)ruthenium(II) complex (4). A mixture of complex **2** (33 mg, 0.026 mmol), 3-(triethoxysilyl)propyl isocyanate (**3**, 7.65 mg, 0.031 mmol), and Et_3N (3 drops) was refluxed in dry THF for 6 h under an N_2 atmosphere. After cooling to 25 °C, the reaction mixture was filtered and the filtrate was concentrated *in vacuo* to afford the silicate precursor **4** (23 mg, 63%) as a brown liquid, which was used for the preparation of the sensing membrane without further purification. FT-IR (KBr) 3300 (N-H), 3000 (C-H), 2220 (NCO), 1700 ($NHCO_2$), 1070 cm^{-1} (C-O). MALDI-TOF MS for $C_{85}H_{75}F_{12}N_7O_5P_2RuSi$: calcd 1693.39, found 1403.49 $[M\text{-}2PF_6]^{2+}$.

Preparation of sensing membranes (M1 and M2). Microscopic slides were used as solid supports onto which the sol-gel was cast. Prior to their use, the surface of the microscope slides was activated by treating it with 1 *N* HF solution for *ca.* 2 min, followed by washing with distilled water and MeOH, and then dried at 25 °C. A sol-gel solution was prepared by mixing tetraethyl orthosilicate (0.67 mL, 3 mmol), ethanol (340 μL, 6 mmol), water (108 μL, 6 mmol) and 0.1 *M* HCl solution (3 μL, 0.3 μmol). For the preparation of the covalent immobilized sol-gel membrane (M1), the silicate derivative **4** (120 μg, 137 μmol) was added to the above sol-gel solution and the mixture was stirred for 4 h at 25 °C. The resulting mixture was spread on the microscope slide and dried at 25 °C for 24 h, after which a glassy membrane was formed, and it was finally dried at 80 °C for 12 h.

For the preparation of the doped sol-gel membrane (M2), $Ru(dpp)_3{\cdot}2PF_6^-$ (1.9 mg, 137 μmol) was added to the above sol-gel solution and the mixture was stirred for 4 h at 25 °C. The resulting mixture was spread on the microscope slide

and dried at 25 °C for 24 h, and finally dried at 80 °C for 12 h.

Measurement of fluorescence intensity. The fluorescence spectra and response data of the sensing membranes were obtained with a 2D fluorescence spectrophotometer (Model F-4500, Hitachi, Japan). The 2D fluorescence spectrophotometer was connected by a 2 m bifurcated liquid light conductor (Lumatec GmbH, Germany) to a dye-immobilized sensor membrane on a glass slide in the port of a 10 mL stainless steel gas flow chamber (Fig. 2). Thus, there was no interference from light outside the chamber. The measurement conditions of the spectrophotometer were as follows: scanning speed, 30,000 nm·min^{-1}; PMT voltage, 950 V; excitation wavelength range, 250-650 nm; emission wavelength range 250-650 nm; excitation and emission slits, 10 nm. The desired concentrations of oxygen in water were obtained by passing pure N_2 and O_2 gas through the chamber using computer-controlled mass flow controllers (Model GFC171, Aalborg, USA) with a constant flow rate of 1 L·min^{-1}.

Leaching experiment. After aging, each sensing membrane on the glass slide was washed several times with $CHCl_3$, and immersed in 100 mL of distilled water in a leaching vessel continuously shaken at room temperature. The membranes were periodically withdrawn, placed into the flow chamber, and their fluorescence intensities were measured. The membranes were then returned to the leaching vessel containing fresh distilled water. The extent of dye leaching was determined by calculating the relative decrease in the fluorescence intensity of each sensing membrane compared with the initial value.

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