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Water-Soluble Osmium Complexes Suitable for use in Luminescence-Based, Hydrogel-Supported Sensors

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Abstract Osmium transition metal complexes are of particular interest in luminescence-based sensing applications because of their longer wavelength absorptions and emissions, relative to similar ruthenium and rhenium complexes. that allow for inexpensive excitation and minimize interferences from autofluorescence when the sensor is used in biological samples. Reported here are the photophysical properties of a series of water-soluble osmium complexes suitable for use in hydrogel-based sensors: [Os(bpy)₂(sulf-dpp)]Cl₂, [Os(phen)₂(sulf-dpp)]Cl₂, [Os(dpp)₂(sulf-dpp)]Cl₂, and [Os(CO)₂Cl₂(sulf-dpp)], where bpy is 2,2'-bipyridine, phen is 1,10-phenanthroline, dpp is 4,7diphenyl-1,10-phenanthroline, and sulf-dpp is bathophenanthrolinedisulfonic acid disodium salt. The family of complexes showed minimal oxygen quenching, making them particularly well-suited for sensing applications in which oxygen concentration varies. Luminescence anisotropy was found to depend more significantly on net dipole moment than hydrodynamic radius of the molecule, and, as expected, excited state lifetime and luminescence anisotropy were highly dependent on the local environment of the reporter molecule. Results obtained for hydrogel-based relative humidity sensors containing [Os(CO)₂Cl₂(sulf-dpp)] and [Os(bpy)₂(sulfdpp)]Cl₂ complexes highlight the significant potential for this class of compounds in a hydrogel-supported luminescencebased sensing approach.

Kristi A. Kneas kneask@etown.edu **Keywords** Osmium transition metal complexes · Luminescence-based sensors · Hydrogel · Luminescence anisotropy

Introduction

Because of their high selectivity and sensitivity, luminescence-based sensors have proven useful for a wide variety of applications including humidity sensing, detection of nitro-based explosives, and detection of ATP in human serum [1-3]. Analyte concentrations have been determined from measured changes in emission intensity, emission wavelength, excited state lifetime, and luminescence anisotropy [4–10]. Despite an extensive body of literature devoted to the development of luminescence-based sensors, there remain some design challenges. Notable are the limited availability of analyte-responsive luminophores and the relative difficulty of engineering new ones [11, 12]. In addition, few sensing compounds are suitable for use in aqueous solution or in the detection of vapor, as required for biological and environmental applications [13–15]. Given that many luminescence-based sensors make use of a luminophore embedded within a polymer matrix, a possible way to address the aforementioned limitations is to shift the analyte sensitivity and selectivity to the polymer matrix, over which there is great synthetic control, and use the luminophore as a secondary reporter. A generalized luminescence-based sensing strategy using an environment-sensitive luminophore embedded within a hydrogel was reported previously to measure relative humidity [1], and the approach could be modified to incorporate a smart hydrogel which responds to analytes such as glucose, lactate, and hydrogen [16–18]. In the approach, the hydrogel swells and contracts with changing analyte concentration, thereby changing the local environment of the luminophore (i.e.,

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To exploit the full potential of this generalized sensing approach, the environment-sensitive luminophore must be optimized. Organic fluorophores like dapoxyl sulfonic acid (DSA), which was used previously [1], are beneficial because of high quantum yields but prove less optimal because of poor photostability and short wavelengths of absorption and emission. The former limits the longevity of a sensor, and the latter presents difficulty in biological applications where autofluorescence is an issue [4]. Since there is no covalent attachment of the fluorophore to the hydrogel network, an added challenge is the loss of fluorophore via leaching when the hydrogel swells and contracts in response to analyte. Because intensity-based sensors are prone to errors resulting from changing concentration of luminophore in the path of exciting light, more desirable would be luminophores that exhibit appreciable changes in emission wavelength, excited state lifetime, or luminescence anisotropy, with the last two having potential for greatest sensitivity. Luminescence anisotropy (r, Eq. 1) is determined from the ratio of emitted polarized light relative to the total light emitted when the sample is excited with polarized light. In Eq. 1, $I_{\scriptscriptstyle \perp}$ is the intensity measured perpendicular to the incident light, and I₁₁ is the intensity measured parallel to the incident light.

$$r = \frac{I_{\parallel} - I_{\perp}}{I_{\parallel} + 2I_{\perp}} \tag{1}$$

Dipole moment and hydrodynamic radius of a complex influence anisotropy because of their respective impacts on photoselective excitation by the polarized light and the extent of depolarization of the emission by rotational diffusion. Solvent viscosity also plays a role, with an increase in viscosity resulting in an increased anisotropy because of reduced rotational diffusion of the emitting compound [19].

As a category of luminescent reporter compounds, transition metal complexes (TMCs), most notably those of the platinum metals of Ru, Os, Re, Rh, and Ir have been widely used [20, 21] and meet the demands of the current application. They are thermally and chemically stable, photochemically robust, and have intense visible absorptions, relatively long excited state lifetimes, large Stokes shifts, and reasonable quantum yields, which afford easier measurement and improved sensitivity [4, 20]. Also significant, the luminescence properties of transition metal complexes, including their environment-sensitivity, emission wavelength, excited state lifetime, and fundamental anisotropy, can be tuned by alteration of the ligands [20, 22]. Osmium complexes are of particular interest in the current work because they typically have longer wavelength absorptions and emissions than other luminescent TMCs, thus allowing inexpensive excitation and minimizing interferences from autofluorescence in clinical samples [5, 20]. In addition they have more limited sensitivity to changes in temperature or oxygen concentration [5, 23].

Osmium complexes similar to those reported here have also proven useful in applications such as solar cells, light emitting diodes, and immunoassays where the value of osmium complexes has been highlighted [24-26]. The utility of osmium complexes as biomolecular probes stems from their long wavelength absorption and emission wavelengths, which minimize interference from autofluorescence in biological systems, and their long excited state lifetimes which are suitable for investigation of DNA dynamics, domain-to-domain motions in proteins, rotational motion of lipid vesicles, and antibody binding in immunoassays [4]. Applications such as these also benefit from the ability to tune the photochemical and photophysical properties of luminescent osmium complexes. [27, 28] To improve probes for use in hydrogelbased sensors, environment-sensitive TMCs must be prepared with high anisotropies and hydrodynamic radii matching the void volume in the hydrogel when swollen over the analyte concentration range of interest. Ligand tuning can also be used to control lipophilicity and hence the partitioning of TMCs between aqueous phase and membranes [29], thus indicating the possibility of positioning TMCs at the hydrogelaqueous solution interface to improve sensitivity in hydrogelbased sensors.

In the work reported here, the goal was to prepare and characterize water-soluble osmium complexes with high fundamental anisotropy, long emission wavelengths, and long excited state lifetimes for use in hydrogel-based sensors. Given the nature of these osmium alpha diimine complexes, a number of these design features are in competition with one another. Figure 1 shows a simplified orbital diagram and typical triplet state orderings for osmium complexes containing alpha-diimine ligands such as 2,2'-bipyridine, 1,10phenanthroline, and 4,7-diphenyl-1,10-phenanthroline. As is the case for the widely utilized family of ruthenium (II) polypyridal complexes, the lowest excited state in osmium complexes of this type is typically a charge transfer state. [20, 30, 31] There are, however, two important differences in the orbital diagram for osmium as compared to that of ruthenium. Crystal field splitting, which increases when descending a group, is significantly greater for Os than for Ru, thereby resulting in significantly higher d-d states for osmium complexes. Resultant benefits are greater photostability and minimal temperature-dependence since the d-d states are thermally inaccessible from the emitting MLCT state. Easier oxidation of osmium relative to ruthenium also means that the energy of the MLCT state is significantly lower than that of analogous ruthenium complexes. A favorable consequence is longer emission wavelengths, but a disadvantage is the increased efficiency of quenching to the ground state and thus reduced quantum yield and shorter excited state lifetimes of these types of osmium complexes, in accordance with the



Fig. 1 Simplified orbital diagram **a** and lowest triplet state orderings **b** for $[Os(L)(L)(L)]^{2+}$ where $L = \alpha$ -diimine ligands such as bipyridine, phenanthroline, and 4,7-diphenyl-1,10-phenanthroline [20, 30]

energy gap law [4, 20]. Modifying ligands to increase the MLCT slightly can thus increase the luminescence intensity of osmium complexes by raising the energy of the MLCT state [4].

In the current work, maximum emission wavelength as well as lifetime and luminescence anisotropy were tuned based on the identity of the ligands and their influence on the MLCT state and the degree of rotational diffusion. In order to elucidate photophysical trends including the emission wavelength and impact of hydrodynamic radius and net dipole moment on luminescence anisotropy, ligands were systematically altered in a set of homologous diimine osmium (II) complexes, as shown in Fig. 2. All complexes contain the bidentate bathophenanthrolinedisulfonic acid disodium salt ligand (sulf-dpp) to ensure water solubility for applications in hydrogel-based sensing. The addition of 2,2'-bipyridine (bpy), 1,10-phenanthroline (phen), or 4,7-diphenylphenanthroline (dpp) allowed for tuning of the MLCT state as well as the inversely related hydrodynamic radius and net dipole moment. The addition of strong crystal field carbonyl ligands was expected to increase the MLCT above that of the tris-substituted polypyridyl complexes, while also allowing the impact of increased dipole moment on luminescence anisotropy to be assessed. Observed trends in photophysical behavior are rationalized, and the potential for this family of complexes in hydrogel-based sensors is illustrated through use of two of the complexes in a hydrogel-based relative humidity sensor.

Experimental

Materials Bathophenanthrolinedisulfonic acid disodium salt hydrate (98 %), ammonium hexachloroosmate (IV) (99.9 %),

bathophenanthroline (98 + %), 1,10-phenanthroline (99 %), and acrylamide (99.9 %) were purchased from Alfa Aesar. Formaldehyde (37 % aqueous solution stabilized with 10-15 % methanol), formic acid (98 %), N,N'-methylene-bis-acrylamide (98 + %), 2-(dimethylamino)ethyl methacrylate (98 %), potassium persulfate (99 + %), and Sephadex G-50 were purchased from Sigma-Aldrich. Ethanol (Certified ACS), acetone (NF grade), and ethyl ether (Reagent grade ACS) were purchased from Pharmco-Aaper, and methanol (Reagent grade ACS) was obtained from Carolina Biological Supply Company. GFS Chemicals was the source of 2,2'-bipyridine (99 %), and sodium hydrosulfite (Certified ACS), ethylene glycol (purified, suitable for histological use) and 2-propanol (ACS Plus) were obtained from Fisher. Nitrogen gas, (ultra high purity), a 50:50 nitrogen:oxygen gas mixture (reagent grade), and oxygen gas (reagent grade) were obtained from Roberts Oxygen Company. Glycerol (ultra-pure) was obtained from MP Biomedicals, and dichloromethane (spectrophotometric grade) was obtained from Acros. With the exception of dichloromethane, which was dried, all reagents were used as received. Hazardous waste was collected for disposal.

Synthesis of $[Os(Phen)_3]Cl_2$ Ammonium hexachloroosmate and 1,10-phenanthroline in a 1:4 M ratio were refluxed in ethylene glycol for 2 h under nitrogen according to literature methods [32, 33]. A color change from burgundy to brown to black was observed. $[Os(phen)_3]Cl_2$ was extracted in a 4:1 ethyl ether:acetone solution (×5), and the dark gray solid was recovered by rotary evaporation in 70 % yield. A single exponential fit to the excited state lifetime decay indicated that no structurally similar luminescent impurities were present.

Synthesis of $[Os(L)_2(Sulf-Dpp)]Cl_2$ The preparation of the homologous series of osmium complexes with alphadiimine ligands was modified from previous literature methods [5]. The precursor $Os(L)_2Cl_2$ was synthesized by refluxing ammonium hexachloroosmate in a 1:2 M ratio with L = 2,2''-bipyridine (bpy), 1,10-phenanthroline (phen), or bathophenanthroline (dpp) in 10 mL of ethylene glycol under nitrogen for 45 min. After cooling to room temperature, sodium hydrosulfite (0.17 M) was added to reduce the osmium from Os(IV) to Os(II). Precipitation of $Os(bpy)_2Cl_2$, $Os(phen)_2Cl_2$, and $Os(dpp)_2Cl_2$ was achieved via an ice-bath, and the solid was washed with deionized water and ethyl ether. Yields were close to 80 %.

 $Os(L)_2Cl_2$ and bathophenanthrolinedisulfonic acid disodium salt hydrate (sulf-dpp) were combined in a 1:1 M ratio with 15 mL of 60:40 solution of ethanol:water and refluxed under nitrogen for 5 h. After full conversion as determined by TLC, the product, $[Os(bpy)_2(sulf-dpp)]Cl_2$, $[Os(phen)_2(sulf-dpp)]Cl_2$, or $[Os(dpp)_2(sulf-dpp)]Cl_2$, was precipitated in acetone in 35 % yield. Conversion was supported by the disappearance of the diagnostic IR peak at 760 cm⁻¹ corresponding to chloride ligands and the appearance of a peak at 1190 cm^{-1} indicating the presence of the sulfonated ligand. A single exponential fit to the excited state lifetime decay indicated that no structurally similar luminescent impurities were present. $[Os(bpv)_2(sulf-dpp)]Cl_2$ ¹H NMR (400 MHz, D_2O): $\delta = 2.66$ [d, 2H, Ar-H], 8.34 [m, 4H, Ar-H], 7.82 [d, 2H, Ar-H], 7.71 [m, 4H, Ar-H], 7.62 [d, 2H, Ar-H], 7.44 [br, 2H, Ar-H], 7.12-7.30 [m, 10H, Ar-H], 6.85 [br, 2H, Ar-H]. IR (KBr pellet) $v(cm^{-1})$: 3448(m), 1638(m), 1394(s), 1194(s), 1125(m), 1034(m), 795(w). $[Os(phen)_2(sulf-dpp)]Cl_2$ ¹H NMR (400 MHz, D₂O): $\delta = 8.88$ [d, 2H, Ar-H], 7.88 [br, 2H, Ar-H], 7.74[[m, 4H, Ar-H], 7.31–7.56 [m, 12H, Ar-H], 7.108[t, 2H, Ar-H], 7.01[br, 2H, Ar-H], 6.97 [br, 2H, Ar-H], 6.84[br, 2H, ar-H]. IR (KBr pellet) $v(cm^{-1})$: 3423(m), 2995(w), 1605(w), 1560(m), 1485(w), 1425(s), 1406(s), 1384(s), 1275(m), 1192(s), 841(s), 733(m), 711(m). [Os(dpp)₂(sulf-dpp)]Cl₂:¹H NMR (400 MHz, D_2O): $\delta = 9.04$ [br, 6H, Ar-H], 7.81–7.87 [m, 20H, Ar-H], 7.72 [br, 6H, Ar-H], 7.63 [m, 14H, Ar-H]. IR (neat) $v(cm^{-1})$: 3448(w), 2997(w), 1619(w), 1572(w), 1430(m), 1416(m), 1396(m), 1226(s), 1181(m), 1075(s), 1026(m), 910(m), 845(s), 828(s), 761(m), 700(s).

Synthesis of [Os(CO)₂Cl₂(Sulf-Dpp)] The synthesis of [Os(CO)₂Cl₂(sulf-dpp)] was modified from a published procedure [34, 35]. The precursor [Os(CO)₂Cl₂] was synthesized

Photophysical Characterization The photophysical properties of the complexes in deionized water, glycerol, and



polypyridyl ligands

(II) complexes with

by refluxing 0.95 mmol ammonium hexachloroosmate with 15 mL of formaldehyde in 40 mL formic acid for three days. The solution progressed in color from red to brown to yellow. The solvent was removed in vacuo, and the product was titurated with dicholoromethane and collected by filtration. The product was dissolved in acetone, NH₄Cl was removed by filtration, and the solvent was removed in vacuo, resulting in a light brown solid of [Os(CO)₂Cl₂] in 50 % yield. [Os(CO)₂Cl₂(sulf-dpp)] was synthesized by refluxing bathophenanthrolinedisulfonic acid disodium salt hydrate and [Os(CO)₂Cl₂] in a 3:2 M ratio for 8 h in 15 mL 2propanol with ~2 mL water added for increased solubility. Conversion to the final product was supported by the decrease in signal of diagnostic IR peaks at 2122 cm⁻¹ and 2012 cm⁻¹ corresponding [Os(CO)₂Cl₂] and the growth of IR peaks at 2136 cm⁻¹, 2037 cm⁻¹, and 1960 cm⁻¹ corresponding to [Os(CO)₂Cl₂(sulf-dpp)]. [25] The solvent was removed in vacuo, and a Sephadex G-50 column with methanol eluent was used to purify [Os(CO)₂Cl₂(sulf-dpp)] for a 50 % yield. ¹H NMR (400 MHz, D_2O): $\delta = 7.34-7.55$ [m, 8H, Ar-H], 7.75 [d, 2H, Ar-H], 7.80 [d, 2H, Ar-H], 8.19 [d, 2H. Ar-H]. IR (KBr pellet) ν (cm⁻¹): 3422(m), 2959(w), 2136(m), 2037(m), 1961(m), 1617(m), 1384(s), 1194(m), 115(w), 1032(m).



Fig. 3 Normalized absorbance (---), excitation (...), and emission spectra (—) of $[Os(bpy)_2(sulf-dpp)]Cl_2$ in water at room temperature

glycerol:water solutions of varying viscosity were measured at room temperature. A Shimadzu recording UV-VIS spectrophotometer (UV-2401PC) was used to obtain absorbance spectra, and a Fluorolog Horiba Jobin Yvon Fluorolog-3 Model FL3-22 spectrofluorometer was used to obtain uncorrected and corrected luminescence excitation and emission spectra, steady state luminescence anisotropy, and excited state lifetime. For spectral acquisition, polarizing filters were used in the excitation and emission paths at 0° and 90°, respectively, in order to minimize contributions from scattered radiation. Anisotropy measurements (8 replicates for spectra and 100 replicates for single wavelength analysis) and lifetime measurements (in triplicate) were acquired using the uncorrected excitation and emission maxima. Excited state lifetimes were acquired via TCSPC using the Horiba Fluorohub-B accessory and DAS6 Fluorescence-Decay Analysis Software; a 450 nm Horiba Scientific NanoLED laser was the source. Four-point Stern-Volmer oxygen quenching plots were obtained from the excited state lifetime measurements (in triplicate) of nitrogen-, air-, 50:50 nitrogen:air-, and oxygensaturated solutions.

Preparation and Calibration of Humidity Sensor The prepolymer solution was prepared as described previously [1] by adding 0.5 mg of [Os(bpy)₂(sulf-dpp)]Cl₂ to 1.3 mL of 40 wt.% acrylamide solution, 1.2 mL of 2 wt.% N-N'- methylenebisacrylamide solution, and 0.4 mL of 2-(dimethyl amino)ethylmethacrylate. The solution was purged under nitrogen with stirring for 20 min prior to the addition of 20 uL of



Fig. 4 Normalized excitation (---)and emission spectra (-- excited at 395 nm; — excited at 500 nm) with anisotropy spectra (\triangle emission at 550 nm; \circ emission at 730 nm; *excitation at 395 nm; x excitation at 500 nm) for [Os(CO)₂Cl₂(sulf-dpp)] in glycerol

a saturated potassium persulfate solution. After stirring for 1 min, 400 uL of the prepolymer solution was delivered via microliter pipet onto a pre-scored, glass microscope slide (Corning, 7.6 cm \times 2.5 cm \times 1 mm), spread uniformly over the surface, and allowed to polymerize at room temperature for 24 h before heating in an oven at 55 °C for 2 h. Six $2.5 \text{ cm} \times 0.8 \text{ cm}$ sensing films were obtained from each microscope slide. The response of resultant sensor films to changing relative humidity was calibrated in flowing gas as described previously [1]. Humidity was varied by controlling the relative flow rates from two nitrogen gas tanks-one dry and one sent through a deionized water bubbler-which were passed into an airtight mixing chamber. Relative humidity was measured at ambient temperature using a Vernier RH-BTA relative humidity probe placed in the mixing chamber just prior to an outlet tube that was directed into the cuvette containing the sensor film in the spectrofluorimeter. Sensor films were preconditioned by cycling back and forth between 10 and 100 % humidity until constant emission response, measured from the front face, was observed.

Results and Discussion

As shown in Fig. 3, the absorption spectrum of $[Os(bpy)_2(sulf-dpp)]Cl_2$ in water at room temperature exhibits

 Table 1
 Maximum absorption

 and corrected emission
 wavelengths of osmium

 complexes
 complexes

	Maximum absorption wavelength (nm)		Corrected maximum emission wavelength (nm)		
	Water	Glycerol	Water	Glycerol	
Os(bpy) ₂ (sulf-dpp)]Cl ₂	435, 480	440, 485	760	750	
Os(phen) ₂ (sulf-dpp)]Cl ₂	435, 480	440, 480	750	740	
Os(dpp) ₂ (sulf-dpp)]Cl ₂	450, 495	450, 495	745	740	
Os(CO) ₂ Cl ₂ (sulf-dpp)]	445, 490	450, 495	730	570, 730	
Os(phen) ₃]Cl ₂	430, 475	435, 480	725	735	

an intense π - π * ligand-centered absorption and a doublehumped MLCT band with maxima at 435 and 480 nm. The absorption peak exhibits a tail extending to 700 nm, thus allowing for the use of low-cost excitation sources. Spectra obtained for [Os(phen)₂(sulf-dpp)]Cl₂ and [Os(dpp)₂(sulfdpp)]Cl₂ showed similar peak shapes with maxima as reported in Table 1 and an extended tail to 700 nm. The observed peak shape is consistent with that obtained in the current work and as previously reported in the literature for [Os(phen)₃]Cl₂ [22, 33].

Excitation spectra for $[Os(bpy)_2(sulf-dpp)]Cl_2$ (Fig. 1) and its analogs exhibit a broad MLCT band with the maximum excitation wavelength corresponding to the shorter wavelength MLCT absorption maximum. In the excitation spectra, the contribution of the 315 nm π - π * ligand-centered peak was more pronounced for these complexes containing the sulfonated dpp ligand as compared to $[Os(phen)_3]Cl_2$ for which the ligand-centered excitation peak was of significantly lower intensity. The relative magnitude of the ligand-centered peak was found to increase from bpy to phen to dpp in the homologous series, consistent with the increasing conjugation of the ligands and stabilization of the excited state.

Wavelengths of maximum emission for the series of $[Os(L)_2(sulf-dpp)]Cl_2$ complexes in aqueous solution (Table 1) decreased from L = bpy to phen to dpp (760, 750, and 745 nm, respectively), indicating an increase in energy of the MLCT excited state resulting from the increase in ligand field strength. Further evidence of the relationship between strength of pi-acceptors and emission wavelength is apparent in the comparison of [Os(sulf-dpp)3]Cl2, which was previously reported in the literature, to [Os(dpp)₂(sulf-dpp)]Cl₂. The addition of sulfonated groups should enhance pi-accepting ability of the dpp ligand, and indeed, the tri-substituted sulfdpp complex exhibited a shorter emission wavelength (731 nm for the complex in 1 M tris HCl, pH 7) [22, 33] than the mono-substituted sulf-dpp complex. When comparing [Os(phen)₃]Cl₂ to [Os(phen)₂(sulf-dpp)]Cl₂, it seems apparent that the decrease in energy of the MLCT state is a consequence of the changing symmetry of the complex as opposed to the addition of a sulfonated dpp ligand.

As shown in Fig. 4, [Os(CO)₂Cl₂(sulf-dpp)] measured in glycerol exhibited two distinct excited states with maximum

emission wavelengths of 570 and 730 nm, measured optimally with 390 nm and 450 nm excitation, respectively. The absorption spectrum was dominated largely by ligand-localized transitions between 250 and 350 nm, but a small MLCT sideband was observed to extend to 650 nm. The higher energy emission is believed to originate from a charge transfer excited state localized on the less readily reduced carbonyl ligands, while the lower energy emission, which is consistent with results obtained for complexes containing alpha-diimine ligands (Table 1), is believed to originate from a charge transfer excited state localized on the sulf-dpp ligand. Both excited states are too low in energy to be attributed to ligandlocalized $\pi \rightarrow \pi^*$ excited states, given that the free sulf-dpp ligand exhibits optimum excitation and emission wavelengths of 340 nm and 390 nm, respectively. Not surprisingly, emission from the higher energy excited state is not observed for the complex in aqueous solution, likely a consequence of rapid internal conversion to the lower energy charge transfer state. Also as expected, the addition of the carbonyl served to increase the energy of the excited state and thus decrease the maximum emission wavelength as compared to the tris polypyridyl complexes (Table 1). Despite the presence of chloride ligands, constant emission intensities indicated that photodecomposition was not a significant issue, likely a consequence of the use of solvents of high dielectric constant and the presence of the alpha diimine ligand [36, 37].

It was anticipated that the excited state lifetimes of the osmium complexes would follow the energy gap law which suggests that excited state lifetimes and quantum yields within a homologous series will decrease exponentially as the MLCT energy is lower and nonradiative processes compete more effectively [4, 22, 38–40]. The bpy complex with the longest emission wavelength indeed exhibited the shortest excited state lifetime, as observed in Table 2 for nitrogen-purged solutions. Slightly longer excited state lifetimes were observed for the phen, dpp, and carbonyl-containing complexes, consistent with their shorter emission wavelengths, but the difference between them is not statistically significant. Likewise, the compounds were equally emissive, exhibiting similar quantum yields that are at least an order of magnitude smaller than [Ru(bpy)_3]Cl_2 [39, 41] but appreciable such that small

 Table 2
 Excited state lifetimes, Stern-Volmer oxygen quenching constants, and bimolecular quenching rate constants for osmium complexes in aqueous solution

	N ₂ (ns)	Air (ns)	50:50 N ₂ :O ₂ (ns)	O ₂ (ns)	$K_{\rm SV}(M^{-1})$	$k_{O} (M^{-1} s^{-1})$	R^2
[Os(bpy) ₂ (sulf-dpp)]Cl ₂	48 ± 3	47 ± 2	43 ± 4	38 ± 3	6.7 ± 0.6	$(1.4 \pm 0.2) \times 10^8$	0.984
[Os(phen)2(sulf-dpp)]Cl2	74 ± 4	73 ± 5	65 ± 5	56 ± 4	8.3 ± 0.1	$(1.1 \pm 0.2) \times 10^8$	0.974
[Os(dpp)2(sulf-dpp)]Cl2	75 ± 4	71 ± 6	56 ± 8	49 ± 5	14 ± 2	$(1.9\pm0.3)\times10^8$	0.958
[Os(CO)2Cl2(sulf-dpp)]	73 ± 5	69 ± 3	63 ± 4	55 ± 5	8.1 ± 0.2	$(1.1\pm0.1)\times10^8$	0.999
[Os(phen) ₃]Cl ₂	78 ± 3	73 ± 4	62 ± 5	52 ± 5	12.6 ± 0.8	$(1.6\pm0.1)\times10^8$	0.993

*uncertainties reported as 95 % C. L

amounts of compound give reasonable signal for the ratiometric types of sensors described here.

Stern-Volmer oxygen quenching constants, K_{SV} , were obtained from four-point Stern-Volmer plots generated from the excited state lifetimes in Table 2 and the Stern-Volmer Eq. (Equation 2)

$$\frac{\tau_o}{\tau} = 1 + K_{SV} \left[O_2 \right] \tag{2}$$

where τ_o and τ are the excited state lifetime in the absence and presence of oxygen, respectively. Bimolecular quenching rate constants, k_o , reported in Table 2 were calculated from the Stern-Volmer quenching constant, which is the product of the biomolecular quenching rate constant and τ_o [42]. As expected for dynamic quenching in solution, plots were highly linear, indicated by the R-squared value. Measured Stern-Volmer quenching constants were quite low as compared to a value of 2130 M⁻¹ for the well-utilized oxygen sensor [Ru(bpy)₃]²⁺ [41], which makes these osmium complexes advantageous in applications where oxygen concentrations are variable. The bimolecular quenching rates constants are within experimental error of one another as expected because the accessibility of the luminophores to the quencher is expected to be roughly the same for this family of complexes [42].

Excited state lifetimes of air-equilibrated aqueous solutions of the tris-substituted polpyridyl complexes exhibit a dependence on the viscosity of the solution and thus rigidity of the local environment of the luminophores, as seen in Table 3. The decrease in excited state lifetime with increasing glycerol concentration observed for complexes in Table 3 is too significant to be attributed solely to a reduction in the Henry's Law solubility of oxygen in glycerol relative to water, particularly given the low quenching constants for these complexes. The low intensity of [Os(CO)₂Cl₂(sulf-dpp)] in glycerol-containing solvents precluded accurate measurement of excited state lifetimes for comparison. With its low Stern-Volmer quenching constant and good environmental sensitivity, [Os(bpy)₂(sulf-dpp)]Cl₂ shows promise as a reporter molecule in hydrogel-based sensors with excited state lifetime as the means of analyte measurement. Shorter excited state lifetimes would be expected as the hydrogel swells in response to a change in analyte concentration.



Fig. 5 Excitation (dotted) and emission (solid) spectra with anisotropy for [Os(bpy)₂(sulf-dpp)]Cl₂ in glycerol

The luminescence anisotropy of the homologous series of osmium complexes was also investigated to determine whether hydrodynamic radius (i.e., size) or net dipole moment has a greater impact. Anisotropy with respect to excitation and emission are shown in Figs. 4 and 5 for [Os(CO)₂Cl₂(sulfdpp)] and [Os(bpy)₂(sulf-dpp)]Cl₂ measured in glycerol. As expected, the emission anisotropy is independent of wavelength for [Os(bpy)₂(sulf-dpp)]Cl₂ because the emission originates from the lowest energy excited state [19]. In the case of [Os(CO)₂Cl₂(sulf-dpp)], the emission anisotropy shows a dependence on wavelength because of the population of the two charge transfer excited states, but, as expected, the anisotropy is constant for a given excited state with the higher energy excited state exhibiting greater anisotropy (Fig. 4). For both complexes, there is an increase in excitation anisotropy with increasing wavelength of excitation.

Table 4 summarizes anisotropy measurements in glycerol and 6:4 glycerol:water solutions for the family of complexes. Not surprisingly, anisotropy decreased significantly upon the addition of water since the molecules rotate more freely in solution. The measured anisotropy values in 6:4 glyercol:water are within experimental of one another. In glycerol, the magnitude of the luminescence anisotropy is observed to decrease for the complexes from substitution of bpy to phen to dpp. This inverse relationship between anisotropy and hydrodynamic radius suggests that dipole moment has the greater effect. Further support of the dependence on dipole moment is evidenced by the high anisotropy of the carbonyl-containing complex and the low anisotropy of the [Os(phen)₃]Cl₂ complex. The highest

Table 3 Excited state lifetimes ofair-equilibrated aqueous solutionsof osmium complexes in waterand glycerol solutionsof varying viscosity

	Excited state lifetime (ns)			
	H ₂ O	6:4 Glycerol:H ₂ O	9:1 Glycerol:H ₂ O	Glycerol
[Os(bpy) ₂ (sulf-dpp)]Cl ₂	47 ± 2	81 ± 5	100 ± 5	133 ± 8
[Os(phen)2(sulf-dpp)]Cl2	73 ± 5	117 ± 5	150 ± 5	179 ± 5
$[Os(dpp)_2(sulf-dpp)]Cl_2$	71 ± 6	112 ± 5	153 ± 5	178 ± 3

*uncertainties reported as 95 % C. L

Table 4Luminescence anisotropy at optimized excitation and emission wavelengths of osmium complexes at 298 K

	6:4 Glycerol: water solutions		Glycerol solutions	Glycerol solutions	
	$\lambda (nm)^a Ex/Em$	Luminescence anisotropy	λ (nm) ^a Ex/Em	Luminescence anisotropy	
[Os(bpy) ₂ (sulf-dpp)]Cl ₂	500/750	0.005 ± 0.003	500/750	0.071 ± 0.004	
[Os(phen) ₂ (sulf-dpp)]Cl ₂	510/740	0.005 ± 0.004	510/740	0.054 ± 0.006	
[Os(dpp) ₂ (sulf-dpp)]Cl ₂	525/740	0.004 ± 0.004	525/740	0.031 ± 0.008	
[Os(CO) ₂ Cl ₂ (sulf-dpp)]	490/730	0.010 ± 0.004	490/730	0.080 ± 0.010	
			500/550	0.277 ± 0.001	
[Os(phen) ₃]Cl ₂	430/725	0.000	430/725	0.028 ± 0.003	

^a Optimum excitation and emission wavelengths were determined from anisotropy versus excitation and anisotropy versus emission spectra for each complex

*Uncertainties reported as 95 % C. L

anisotropy overall is observed for $[Os(CO)_2Cl_2(sulf-dpp)]$ when excited at 500 nm, thus suggesting that this as the most useful luminophore for application in hydrogel-based sensors where a change in anisotropy would be expected upon swelling and contracting of the hydrogel in response to analyte.

In order to demonstrate proof-of-concept for use in hydrogel-based sensors, [Os(bpy)₂(sulf-dpp)]Cl₂ and [Os(CO)₂Cl₂(sulf-dpp)] were embedded in hydrogel films for use as humidity sensors similar to those reported previously with dapoxyl sulfonic acid as the environment-sensitive reporter molecule. [1] Because anisotropy was observed to change more appreciably than excited state lifetime with changing rigidity of the local environment of these luminophores, luminescence anisotropy was monitored as a function of percent relative humidity. Figure 6 shows pooled anisotropy measurements for three independently prepared sensing films containing [Os(bpy)₂(sulf-dpp)]Cl₂. Film-to-film reproducibility was reasonable, and it was observed that as the hydrogel swelled in response to an increase in relative humidity, there was a concomitant increase in measured anisotropy. This is attributed to the increased solvation and thus decreased rotational diffusion of the reporter molecule with swelling of the hydrogel and uptake of water vapor. The 1.3-fold change in anisotropy shown in Fig. 6 is superior to the sensitivity of our wavelength-based sensor reported previously, which exhibited only a 15 nm wavelength shift over the full humidity range. [1]

Figure 7, which shows results obtained for a sensor film containing [Os(CO)₂Cl₂(sulf-dpp)] as the reporter molecule illustrates several important points. Most significantly, the anisotropy is observed to decrease with increasing relative humidity. This is a consequence of the fact that anisotropy was monitored for the higher energy charge transfer excited state which is likely to be more highly populated when the hydrogel is contracted and the complex is in a rigid environment. There is no change to anisotropy until the relative humidity reaches about 40 %, suggesting that this is the point at which internal conversion to the lower energy charge transfer state begins to play a significant role. While dynamic range is thus limited for this particular sensor to between 40 and 100 % RH, it seems likely that through optimization of hydrogel composition and, more specifically, pore size, it should be possible to control dynamic range. Error bars obtained in Fig. 7 are sizable, as is the case for most anisotropy calibrations obtained for a single hydrogel sensing film of this type. Poor precision is attributed, in part, to the heterogeneity of the reporter molecule within the sensor films. A similar effect is observed for luminescence-



Fig. 6 Pooled anisotropy response as a function of percent relative humidity for three acrylamide/2-(dimethyl amino)ethylmethacrylate sensing films with $[Os(bpy)_2(sulf-dpp)]^{2+}$ as the reporter molecule



Fig. 7 Anisotropy as a function of percent relative humidity for an acrylamide/2-(dimethyl amino)ethylmethacrylate sensing film with $[Os(CO)_2Cl_2(sulf-dpp)]$ as the reporter molecule

based oxygen sensing films, particularly when there is poor luminophore-polymer compatibility [43]. Modification of the hydrogel composition to improve compatibility could address this limitation, and it is also likely that improved deposition methods could produce films of higher optical quality, thus minimizing any error associated with scattered radiation. No apparent hysteresis is observed for calibrations, and the response is fully reversible as evidenced by the similarity of measurements made upon both increasing and decreasing relative humidity. The greater than 3-fold change in anisotropy observed for this sensor in the 40–100 % RH range is quite good.

Conclusion

The osmium complexes reported here, and particularly [Os(bpy)₂(sulf-dpp)]Cl₂ and [Os(CO)₂Cl₂(sulf-dpp)], show great promise as reporter molecules in hydrogel-based sensors or any sensing scheme requiring a change in emission response upon change in local environment of the reporter molecule. Distinct advantages include their water solubility, the ability to make use of longer wavelength excitation as compared to their ruthenium and rhenium counterparts, and the opportunity to eliminate sources of error typically associated with intensity-based measurements through use of ratiometric anisotropy or intensity-independent excited state lifetime measurements. In addition, the tailorability of the osmium complexes in terms of hydrodynamic radius, dipole moment, and photophysical properties should allow further optimization for particular sensing applications with a given support matrix. Likely, greatest sensitivity would be achieved in a hydrogelbased sensor when the pore size in the contracted hydrogel is closely matched to the hydrodynamic radius of the complex. Under such circumstances, the complex would experience a largely hydrophobic environment when the hydrogel is contracted, and even a very small degree of swelling should result in a significant change in the polarity of the local environment. As the results here have shown, it is possible to control the dynamic range of a sensor by varying the reporter molecule, thus providing a means by which to increase sensitivity over a narrow range of interest.

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