J|A|C|S
COMMUNICATIONS

Iridium Doped Silica−PEG Nanoparticles: Enabling Electrochemiluminescence of Neutral Complexes in Aqueous Media

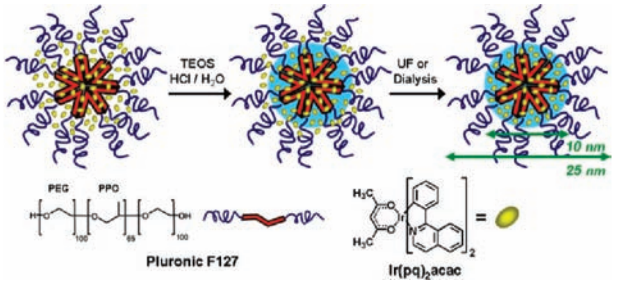
Simone Zanarini,* Enrico Rampazzo,* Sara Bonacchi, Riccardo Juris, Massimo Marcaccio, Marco Montalti, Francesco Paolucci,* and Luca Prodi*

Dipartimento di Chimica “G. Ciamician”, Università di Bologna, Via Selmi 2, 40126 Bologna, Italy

Received August 14, 2009; E-mail: simone.zanarini@unibo.it; enrico.rampazzo@unibo.it

Electrochemiluminescence (ECL) is superior to photoluminescence (PL) in terms of sensitivity and intrinsically low noise.[1] Nowadays almost only Ru(bpy)$_3^{2+}$ based ECL labels are used in bioanalysis.[1d,e,h] In fact, although many Ir(III) neutral complexes are known to be ECL active in a nonaqueous environment[2] often with a degree of efficiency much higher than Ru(bpy)$_3^{2+}$, their use has been restricted by their very low solubility and sensitivity to oxygen quenching. Biological systems have been supremely successful in overcoming the limitations of aqueous media by developing complex, organized structures at the nanoscale level. Similarly, Dye Doped Silica Nanoparticles (DSN), which conjugate crucial features such as low toxicity, simple preparation and low cost, are a possible approach to create water-soluble multifunctional systems,[3] providing the means for dramatically improving the performance of ECL labels. We report here for the first time the ECL emission of Ir(pq)$_2$acac-doped silica nanoparticles (Ir-DSN) in aqueous buffer solutions.

Scheme 1. Schematic Structure and Preparative Steps of Ir-DSN



In particular, we implemented the direct micelles assisted method[4] to obtain core−shell/silica−polyethyleneglycol nanoparticles. We found that this strategy can be very versatile for the preparation of stable luminescent nanoparticles doped with highly hydrophobic materials. With a low doping degree in fact, a number of luminescent guest molecules can be irreversibly hosted into the inner compact nanoparticles silica core (see Scheme 1). These dye doped nanoparticles are made of a silica core and a PEG (polyethyleneglycol) outer shell able to confer sterical stabilization to the particles.[5] Ir(pq)$_2$acac (see Scheme 1 and Figure S1 in the Supporting Information (SI)) was selected as the doping material mostly due to the highly intense ECL emission in acetonitrile/tripropylamine (TPA) solutions.[2i] After Ir-DSN preparation, the irreversible inclusion of the dye was proved by means of extensive ultrafiltration[6] or dialysis experiments. Moreover, extraction procedures carried out in a water/2-octanol system were unsuccessful in the removal of the lipophilic Ir(pq)$_2$acac dye from the aqueous nanoparticles solution, excluding the presence of the iridium complex in the PEG stabilizing shell. In addition, the high polarization value ($r = 0.15$) of the luminescence found for the nanoparticles offers further support for the localization of the luminophores in the inner rigid silica core (see SI).[7]

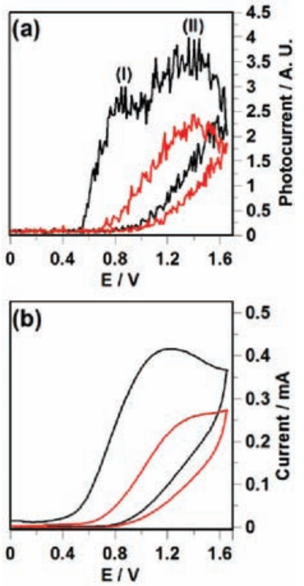


Figure 1. Light (a) and current (b) potential profile of a Ir(pq)$_2$acac doped silica nanoparticles solution in phosphate buffer 0.3 M containing 30 mM DBAE. The ECL emission was registered during a CV with $E_1 = 0$ V, $E_2 = +1.6$ V and scan rate = 0.2 V/s. Working electrode: 2 mm diameter Pt disk. Potentials are vs Ag/AgCl. The colors black and red indicate the first and second voltammetric cycle, respectively.

Hydrodynamic diameter distribution was determined by Dynamic Light Scattering (DLS) revealing a value of ~25 nm (see Figure S2 in the SI), while a silica core diameter of ~10 nm for the Ir-DSN was estimated by trasmission electron microscopy (TEM) (Figures S3 and S4 in the SI) and atomic force microscopy (AFM) measurements (Figures S8 and S9 in the SI). The AFM imaging and analysis of the tapping mode and phase-imaging mode show the morphology of the nanoparticles, highlighting the different viscoelastic properties of the soft PEG-shell and the harder silica core, as analogously observed in hybrid organic−inorganic nanocomposites.[8] The stability of the nanoparticles solution was monitored for up to 6 months after preparation, revealing morphological features and photophysical properties unaltered.

The Ir-DSN absorbance and photoluminescence spectra were similar to those of free Ir(pq)$_2$acac in methanol. The luminescence quantum efficiency of Ir(pq)$_2$acac inside Ir-DSN is enhanced by ~30% with respect to the free dye in an aerated methanol solution as shown by the long average lifetime found (1380 ns), a value very close to the one obtained for the complex in a deaerated methanol solution (1545 ns vs 72 ns in aerated solutions; see Table S1 in the SI).

10.1021/ja906666e CCC: $40.75 © 2009 American Chemical Society

To perform a preliminary electrochemical characterization 200 μL of the Ir-DSN prepared solution were diluted with 100 μL of a 1 M phosphate buffer (pH 7.5). Thus, the Ir-DSN concentration was kept as high as possible.[9]

The cyclic voltammogram of such a solution did not show any oxidation process before solvent discharge. The oxidation of Ir(III) inside silica nanoparticles, as $Ru(bpy)_3^{2+}$, was not electrochemically visible[10] because of the concentration of the complex ($\sim 10^{-5}$ M) and the low diffusion coefficient of the nanoparticles.[10] The ECL emission was generated by using 2-(dibutylamino)ethanol (DBAE) oxidative coreactant. This compound was preferred to tripropylamine because it is less toxic, is more water-soluble, and gives a more intense ECL signal.[11] DBAE was added to the Ir-DSN solution at 30 mM concentration. The typical synchronized light/current/potential profile is shown in Figure 1. The voltammetric profile (Figure 1b) is dominated by the irreversible oxidation of DBAE[11] and, eventually, by a residual amount of chloride.

The ECL intensity vs potential (Figure 1a) shows interestingly two maxima; the first (marked I) of minor intensity is observed in correspondence to DBAE oxidation, i.e. at $\sim +0.85$ V vs Ag/AgCl. The second maximum of higher intensity falls at $\sim +1.35$ V. The peak is very broad and positively shifted with respect to the $Ir(pq)_2acac$ oxidation potential in MeCN. ($\Delta V \approx +0.1-0.2$ V).[2i] On the other hand, the relatively intense emission and the potential dependence are a clear demonstration that electron hopping occurs even if Ir(III) centers are confined in a silica insulating matrix.[10] The ECL spectrum of Ir-DSN (Figure 2) is coincident with the photoluminescence spectrum of the $Ir(pq)_2acac$ complex in solution (Figures S5 and S6 in the SI), allowing us to unquestionably assign the ECL light emission to the $Ir(pq)_2acac^*$ species.

The route of excited state generation is compatible with that currently established for the TPA/$Ru(bpy)_3^{2+}$ system.[1,10] Two different mechanisms are active in correspondence to the DBAE and $Ir(pq)_2acac$ oxidation potential according to Scheme S1 (in the SI).

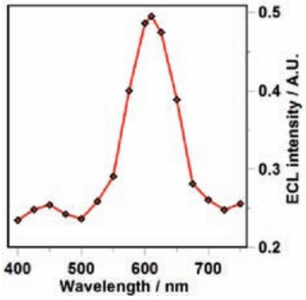


Figure 2. ECL spectra of Ir-DSN in phosphate buffer 0.3 M solution containing 30 mM DBAE. Emission was registered at 25 nm interval during double step potential program: conditioning potential: −1.0 V for 15 s, $E_1 = +1.8$ V (vs Ag/AgCl) for 3 s. PMT bias 750 V.

By comparing the light profile during the first (Figure 1a, black line) and second scan (red line), it is clear that while in the former the two mentioned mechanisms are active, in the latter only direct $Ir(pq)_2acac$ oxidation occurs. This behavior can be understood by observing the corresponding current profiles (Figure 1b) that show a neat area decrease in the second cycle. The inactivation of the lower potential mechanism is associated with passivation of the electrode surface with either Pt oxides or DBAE electrogenerated filming products easily removable by cathodic preconditioning.

In conclusion, we have demonstrated the possibility to obtain stable ECL emissions in aqueous solutions from completely an insoluble neutral Ir(III) complex inside silica−PEG nanoparticles. Considering the flexibility of the silica−PEG nanoparticles architecture, this is a promising way to obtain a great number of water-soluble ECL materials. We are now applying the methodology to a number of Ir(III) complexes and hydrophobic organic emitters to enhance ECL emission intensity and tune effectively the color of these high-potential labeling materials.

Acknowledgment. The authors would like to thank Leopoldo Della Ciana (Cyanagen srl) for supplying the $Ir(pq)_2acac$ complex. The financial support from MIUR (LATEMAR FIRB and PRIN projects) and Fondazione Cassa di Risparmio in Bologna is also gratefully acknowledged.

Supporting Information Available: Starting materials; ultrafiltration and dialysis experiments; nanoparticles synthesis; particles size distribution; TEM experiments; photophysical measurements; electrochemical and ECL experimental setup; AFM experiments; scheme of the mechanisms of excited state generation. This material is available free of charge via the Internet at http://pubs.acs.org.

References

(1) (a) Gerardi, R. D.; Barnett, N. W.; Lewis, S. W. *Anal. Chim. Acta* **1999**, *378*, 1. (b) Knight, A. W. *Trends Anal. Chem.* **1999**, *18* (1), 47. (c) Fahnrich, K. A.; Pravda, M.; Guilbault, G. G. *Talanta* **2001**, *54*, 531. (d) *Electrogenerated chemiluminescence*; Bard, A. J., Ed.; Marcel Dekker: New York, 2004. (e) Richter, M. M. *Chem. Rev.* **2004**, *104*, 3003. (f) Yin, X.-B.; Dong, S.; Wang, E. *Trends Anal. Chem.* **2004**, *23* (6), 432. (g) Gorman, B. A.; Francis, O. S.; Barnett, N. W. *Analyst* **2006**, *131*, 616. (h) Miao, W. *Chem. Rev.* **2008**, *108*, 2506. (i) Wei, H.; Wang, E. *Trends Anal. Chem.* **2008**, *27* (5), 447.

(2) (a) Kapturkiewicz, A.; Nowacki, J.; Borowicz, P. *Z. Phys. Chem.* **2006**, *220*, 525. (b) Kapturkiewicz, A.; Chen, T.-M.; Laskar, I. R. *Electrochem. Commun.* **2004**, *6*, 827. (c) Kapturkiewicz, A.; Nowacki, J.; Borowicz, P. *Electrochim. Acta* **2005**, *50*, 3395. (d) Avilov, I. V.; Minoofar, P.; Cornil, J.; De Cola, L. *J. Am. Chem. Soc.* **2007**, *129*, 8247. (e) Orselli, E.; Kottas, G. S.; Konradsson, A. E.; Coppo, P.; Fröhlich, R.; De Cola, L.; van Dijken, A.; Buechel, M.; Boerner, H. *Inorg. Chem.* **2007**, *46*, 11082. (f) Orselli, E.; Albuquerque, R.; Fransen, P. M.; Fröhlich, R.; Konradsson, A. E.; Janssen, H. M.; De Cola, L. *J. Mater. Chem.* **2008**, *18* (38), 4579. (g) Ragni, R.; Plummer, E. A.; Brunner, K.; Hofstraat, J. W.; Babudri, F.; Farinola, G. M.; Naso, F.; De Cola, L. *J. Mater. Chem.* **2006**, *16*, 1161. (h) P. Coppo, P.; Duati, M.; Kozhevnikov, V. N.; Hofstraat, J. W.; De Cola, L. *Angew. Chem., Int. Ed.* **2005**, *44*, 1806. (i) Kim, J. I.; Shin, I.-S.; Kim, H.; Lee, J.-K. *J. Am. Chem. Soc.* **2005**, *127*, 1614. (j) Shin, I.-S.; Kim, J. I.; Kwon, T.-H.; Hong, J.-I.; Lee, J.-K.; Kim, H. *J. Phys. Chem. C* **2007**, *111* (5), 2280. (k) Stagni, S.; Colella, S.; Palazzi, A.; Valenti, G.; Zacchini, S.; Paolucci, F.; Marcaccio, M.; Albuquerque, R. Q.; De Cola, L. *Inorg. Chem.* **2008**, *47* (22), 10509.

(3) (a) Burns, A.; Ow, H.; Wiesner, U. *Chem. Soc. Rev.* **2006**, *35*, 1028. (b) Yan, J.; Estévez, M. C.; Smith, J. E.; Wang, K.; He, X.; Wang, L.; Tan, W. *Nano Today* **2007**, *2*, 44.

(4) Huo, Q.; Liu, J.; Wang, L. Q.; Jiang, Y.; Lambert, T. N.; Fang, E. *J. Am. Chem. Soc.* **2006**, *128*, 6447.

(5) See Supporting Information for further details.

(6) Rampazzo, E.; Bonacchi, S.; Montalti, M.; Prodi, L.; Zaccheroni, N. *J. Am. Chem. Soc.* **2007**, *129*, 1425.

(7) (a) Lackowicz, J. R. *Principles of fluorescence spectroscopy*; Springer: New York, 2006. (b) Birch, D.; Geddes, C. D. *Phys. Rev. E* **2000**, *62* (2), 2977.

(8) (a) Magonov, S. N.; Elings, V.; Whangbo, M.-H. *Surf. Sci.* **1997**, *375*, L385. (b) Fang, F. Z.; Xu, Z. W.; Dong, S. *Meas. Sci. Technol.* **2008**, *19*, 055501. (c) Mazzocchetti, L.; Sandri, S.; Scandola, M.; Bergia, A.; Zuccheri, G. *Biomacromolecules* **2007**, *8*, 672.

(9) To prevent Ir-DSN self aggregation, the concentration of the prepared solution cannot be further increased.

(10) Zanarini, S.; Rampazzo, E.; Della Ciana, L.; Marcaccio, M.; Marzocchi, E.; Montalti, M.; Paolucci, F.; Prodi, L. *J. Am. Chem. Soc.* **2009**, *131* (6), 2260.

(11) Liu, X.; Shi, L.; Niu, W.; Li, H.; Xu, G. *Angew. Chem., Int. Ed.* **2007**, *46*, 421.

JA906666E