Fluorescence microscopy coupled to electrochemistry: a powerful tool for the controlled electrochemical switch of fluorescent molecules†

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The first-time coupling of fluorescence microscopy with a three-electrode electrochemical cell is described and applied to the investigation of a controlled-potential redox switch of organic fluorophores such as tetrazine derivatives.

Redox switching of fluorescence is a very attractive field of research due to the high interest in designing electrically driven reversible luminescent sensors. Although several examples exist in the literature in which the fluorophore emission can be switched by changing the redox state of an active site connected to it,[1–4] the cases where the same moiety acts both as the light emitter and the redox switch are much rarer, especially in the field of organic dyes. Actually to this day only two examples are described,[5,6] one of them implying a multi-step process with a molecular internal rearrangement.[5] Tetrazine derivatives that are currently developed in our group constitute a family of very promising candidates for such applications. Among these compounds, chloromethoxytetrazine exhibits a long fluorescence lifetime in the visible range along with a reversible redox switch between its neutral and anion radical forms at a rather high potential, with little changes in the molecular geometry.[7] An all solid-state electrofluorochromic device was recently designed, demonstrating the ability to reversibly switch the emission properties according to the voltage applied to two electrode plates between which this dye was incorporated as the cathodic material.[8] A further challenge now consists in providing experimental evidences that the luminescence quenching is actually due to the redox switch between the neutral and reduced forms of the tetrazine moiety, which requires the use of a conventional three-electrode cell.

A few examples of experimental set-ups coupling fluorescence and electrochemistry are described in the literature: they allow either photochemical processes to be monitored by electrochemistry[9] or conversely electrochemically induced concentration profiles to be probed by fluorescent sensors[10] or finally simultaneous real-time recording of electrochemical and fluorescence signals.[11] Nevertheless to our knowledge, the direct use of a controlled electrode potential to generate a fluorescence switch has been described only once.[6] The difficulty arises from the possible evolution of the reduced form during timescales longer than those used in cyclic voltammetry, when dealing with electrolysis cells. This problem can be circumvented by using specially tailored spectroelectrochemical cells working in thin layer mode.[6,12,13] With the configuration proposed by Levillain and co-workers[6] the probed distance from the electrode surface can be varied between 20 and 250 μm thanks to a micrometer screw, which is compatible with the diffusion layer thickness in a classical cyclic voltammetry experiment. We present in this communication an alternative set-up allowing us to work in an open-space cell. This is performed by coupling a three-electrode cell in which the working electrode is made of a platinum thin layer coated on a microscope glass slide, with a scanning-less imaging fluorescence set-up[14] (which is equivalent to a microscopy epifluorescence set-up[11]): with such a configuration, it is possible to probe the variation of fluorescence intensity localized in the diffusion layer of an electrode whose potential is controlled. Moreover this set-up allows one to measure the evolution of excited-state lifetimes during the potential switch. The results obtained by recording simultaneously the fluorescence intensity and the coulombic charge during a controlled potential reduction of chloromethoxytetrazine are presented and discussed.

Fig. 1 displays the experimental set-up used to record the fluorescence intensity in the diffusion layer of an electrode whose potential is electrochemically controlled. Fluorescence microscopy allows us to record the fluorescence intensity ($\lambda > 515$ nm) in the vicinity of the slot etched within the platinum layer coated on the microscope glass slide (see ESI†). Lifetime imaging can also be recorded through the QA imaging detector.

The cyclic voltamogram recorded with this set-up for chloromethoxytetrazine is shown in Fig. 2. This enables us to check the accurate potential values required for the electrochemical conversion from the neutral form to the first reduced state (anion radical) of the tetrazine compound. The redox system investigated is fully reversible despite the distortion due to uncompensated ohmic drop.‡

Fig. 3 shows the simultaneous recording of fluorescence intensity and coulombic charge upon multiple potential steps from 0 to −1.2 V and back to 0 V (five cycles). One can see clearly that the reduction of chloromethoxytetrazine to its

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† Electronic supplementary information (ESI) available: 1: Pictures of the epifluorescence microscope set-up and electrochemical cell. The inset shows the picture of the slot within the platinum layer. 2: Fluorescence intensity recorded upon a double potential step from 0 to −0.2 V and reverse during 25 s each. See DOI: 10.1039/b718899d

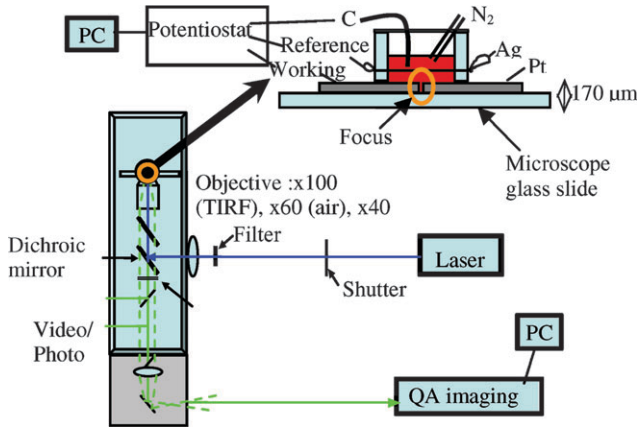

Fig. 1 Experimental set-up for the coupling of epifluorescence microscopy imaging with a three-electrode electrochemical cell (volume: *ca.* 5 mL) connected to a potentiostat (CH Instruments). Excitation beam, $\lambda = 515$ nm (blue), and fluorescence beam (green). The microscope is focused on a thin slot (see ESI†) etched in the platinum layer coated on the microscope glass slide (170 µm thick).

anion radical form induces a regular decrease of emission intensity in the diffusion layer of the working electrode and that the fluorescence emission is almost fully recovered when the potential is switched back, although the coulombic charge remains partially cathodic at the end of the cycle. A likely explanation for this latter point may arise from the existence of an irreversible reduction process of residual oxygen gas along with the tetrazine reduction resulting in unrecovered charge upon the reverse potential step. The system can be switched reversibly between the "on" (oxidized) and "off" (reduced) states during several cycles, without noticeable loss of overall intensity. The same experiment was also performed upon potential steps between 0 and −0.2 V, *i.e.* that do not induce the tetrazine reduction, leading to a perfectly stable fluorescence intensity (see ESI†). This allows us to exclude fluorescence variations due to bleaching phenomena during the potential signal application (note that the laser power on the sample is 50 µW). Finally Fig. 4 displays the Stern–Volmer plot of $I_{f,max}/I_f$ *vs.* charge (I_f: fluorescence intensity) associated with the potential step from 0 to −1.2 V during 60 s. The linear variation observed agrees well with a quenching mechanism

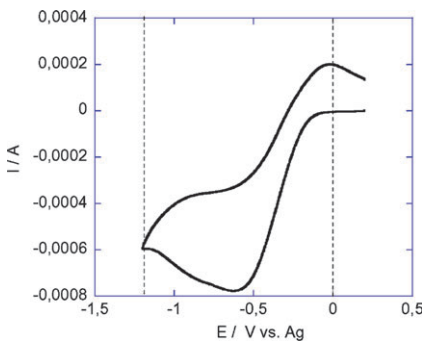

Fig. 2 Cyclic voltamogram recorded with the set-up described in Fig. 1 for chloromethoxytetrazine *ca.* 5 mM in dichloromethane (scan rate: 0.1 V s^{-1}). The dashed lines show the limits of the potential step used to record the signals of Fig. 3.

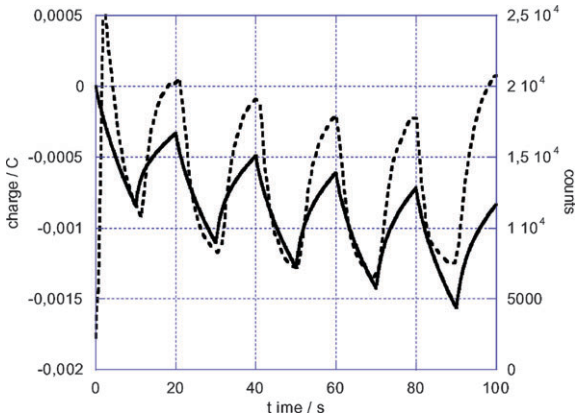

Fig. 3 Simultaneous recording of fluorescence intensity (dashed line) and coulombic charge (full line) upon multiple potential steps from 0 to −1.2 V and reverse (five cycles). Same experimental conditions as in Fig. 2.

that is due to the interaction of the electrogenerated reduced species with the fluorescent tetrazine, the concentration of which is proportional to the injected coulombic charge.[15]

All these results confirm that the fluorescence modulation according to applied voltage recorded in the all-solid fluoroelectrochromic cell[8] is actually correlated to the production of the non-emissive one-electron reduced form of the fluorescent tetrazine dye.

A new set-up coupling a three-electrode electrochemical cell with epifluorescence microscopy has thus allowed us to demonstrate that the conversion of a tetrazine derivative from the neutral to the anion radical state led to a concomitant fluorescence quenching. This fluorescence is fully recovered when the applied potential is reversed. The same set-up can also give valuable information from lifetime imaging and *in situ* absorption measurements along with the electrochemical signal, which makes it a very powerful tool for characterizing the emission properties of fluorescent and redox active molecules. Additional measurements involving excited lifetime imaging are in progress to further investigate the quenching mechanism associated to the recorded variations of fluorescence intensity.

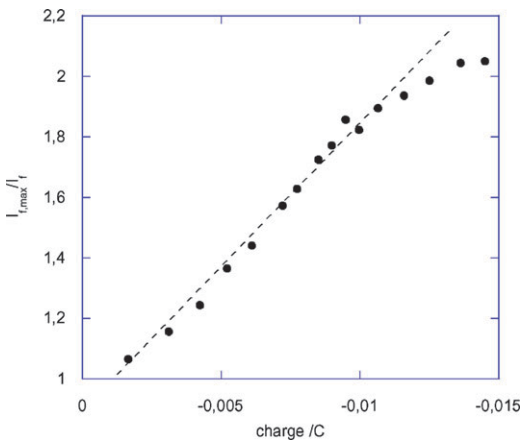

Fig. 4 Stern–Volmer plot of $I_{f,max}/I_f$ *vs.* coulombic charge recorded during a potential step from 0 to −1.2 V for 60 s.

Notes and references

‡ The formation of the anion-radical of the chloromethoxytetrazine has been shown to be totally reversible in several occurrences, and is still in our cell. In the CV displayed, the excess cathodic current arises from traces of oxygen which are difficult to eliminate given the cell configuration and the use of volatile dichloromethane.

1 H. Röhr, C. Trieflinger, K. Rurack and J. Daub, *Chem.–Eur. J.*, 2006, **12**, 689–700.
2 H. Li, J. O. Jeppesen, E. Levillain and J. Becher, *Chem. Commun.*, 2003, 846.
3 R. A. Illos, E. Harlev and S. Bittner, *Tetrahedron Lett.*, 2005, **46**, 8427–8430.
4 R. A. Illos, D. Shamir, L. J. W. Shimon, I. Zilbermann and S. Bittner, *Tetrahedron Lett.*, 2006, **47**, 5543–5546.
5 T. Suzuki, A. Migita, H. Higuchi, H. Kawai, K. Fujiwara and T. Tsuji, *Tetrahedron Lett.*, 2003, **44**, 6837–6840.
6 M. Dias, P. Hudhomme, E. Levillain, L. Perrin, Y. Sahin, F. X. Sauvage and C. Wartelle, *Electrochem. Commun.*, 2004, **6**, 325–330.
7 P. Audebert, F. Miomandre, G. Clavier, M. C. Vernières, S. Badré and R. Méallet-Renault, *Chem.–Eur. J.*, 2005, **11**, 5667–5673.
8 Y. Kim, E. Kim, G. Clavier and P. Audebert, *Chem. Commun.*, 2006, 3612–3614.
9 S. M. Fonseca, S. Ahmed, T. J. Kemp and P. R. Unwin, *Photochem. Photobiol. Sci.*, 2003, **2**, 98–103.
10 F. V. Loëte, B. R. Oltra, D. Chaumont and E. Bourillot, *Electrochem. Commun.*, 2006, **8**, 1016–1020.
11 C. Amatore, S. Arbault, Y. Chen, C. Crozatier, F. Lemaitre and Y. Verchier, *Angew. Chem., Int. Ed.*, 2006, **45**, 4000–4003.
12 I. Yilmaz, *New J. Chem.*, 2008, **1**, 37–46.
13 M. Mukaigawa and H. Ohno, *J. Electroanal. Chem.*, 1998, **452**, 141–149.
14 J.-A. Spitz, R. Yasukuni, N. Sandeau, M. Takano, J.-J. Vachon, R. Méallet-Renault and R. B. Pansu, *J. Microsc.*, 2008, **229**, 104–114.
15 F. Montilla and R. Mallavia, *J. Phys. Chem. B*, 2006, **110**, 25791–25796.