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Coupling thin layer electrochemistry with epifluorescence microscopy: An expedient way of investigating electrofluorochromism of organic dyes

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ABSTRACT

The first example of thin layer electrochemistry coupled to epifluorescence microscopy in the total internal reflectance mode is described and applied to the investigation of electrochemically modulated fluorescence of an organic dye (chloromethoxytetrazine) in solution. This technique allows to generate full redox switch of fluorescence when converting reversibly the dye into its anion radical, as well as to record the spectral features of the electrogenerated species. Recording simultaneously fluorescence intensity and lifetime along with coulombic charge as a function of the electrode potential will lead to a deep insight into the redox quenching mechanism.

1. Introduction

The coupling of epifluorescence microscopy with an electrochemical cell has proven recently to be a very efficient tool for investigating properties of surface confined species [1,2]. Especially the Total Internal Reflectance Fluorescence (TIRF) mode allows to increase the resolution up to the single object detection [3,4], being thus capable of gathering information about electron transfer thermodynamics and kinetics on a very local scale [5]. Combined application of the techniques to molecular systems in a solution has been less common, albeit very interesting for electrochemical monitoring of luminescence properties of dyes, as shown by Ackermann et al. and our group [6–8]. In such a case the major issue to overcome is to get rid of the response coming from the molecules outside the diffusion layer. A simple solution can be provided using a thin layer electrochemical cell, as documented by the pioneering work of Levillain et al. [9]. Due to the exhaustive electrolysis of the compound in the thin solution layer, a full switch of fluorescence (down to zero intensity) can be achieved, contrary to the semi-infinite diffusion configuration [6,7]. Provided that the electrogenerated species is sufficiently stable on the time scale of the in situ electrolysis, all its spectroscopic characteristics can be determined. In this communication, we provide the first example of coupling a versatile thin layer electrochemical cell with epifluorescence microscopy in the TIRF configuration and describe how it can be applied to the investigation of electrochemically monitored fluorescence of an organic dye in solution. We have chosen a tetrazine derivative which is both electroactive in an easily accessible potential range and fluorescent in the visible domain. However, the application of the experimental technique can be extended to any other system fulfilling these features.

2. Experimental set-up

Chloromethoxytetrazine (ClTzOMe) was synthesized according to the literature procedure [10]. The relevant spectroscopic and redox properties of this dye can be found in previously published papers [10,11].

UV–vis spectroelectrochemistry was carried at 293 and 238 K, using an optically transparent thin-layer electrochemical cell (OTTLE) [12] and its cryostatted version [13,14] positioned in the sample compartment of a Scinco S3100 diode array spectrophotometer. The working electrode (Pt minigrid, 32 wires per cm) potential was controlled with a PA4 potentiostat (Laboratory devices, Polná, Czech Republic).

The epifluorescence microscope set-up has been described elsewhere as well as the parameters for pulse laser excitation (wavelength : 515 nm) [8]. The OTTLE cell [12] was placed on the microscope stage and connected to a potentiostat (CHInstruments 600). The laser beam was focused into a thin solution layer in the small quadratic area defined by the Pt wires of the minigrid working electrode (see Fig. 2). Platinum and silver wires were used respectively as counter and pseudo-reference electrodes. The dye concentration was varied from 0.6 to

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1.5 mmol dm^{-3} in dry dichloromethane with tetrabutylammonium hexafluorophosphate (TBAH) as the supporting electrolyte. The solutions were deaerated by argon bubbling prior to each experiment.

DFT calculations were done with the Gaussian 03 software [15]. The geometries of ClTzOMe and its radical anion were optimized at the B3LYP/6–31 + g(d) level of theory. Vertical electronic transitions were calculated by the time dependant (TD) methodology using the PBE0 mixed functional and the 6–311 + g(d,p) basis set [16]. The solvent effect (dichloromethane) was taken into account with the IEFPCM model [17]. Spectra have been obtained with GaussSum 2.2 (FWHM = 2300 cm^{-1}) [18].

3. Results and discussion

Fig. 1 shows the spectroelectrochemical features for the conversion of ClTzOMe into its anion radical within the OTTLE cell. Absorption spectra (Fig. 1A,B) display two main absorption bands of the neutral form at 330 and 520 nm belonging to $\pi-\pi^*$ and $n-\pi^*$ transitions respectively, that gradually disappear while new composed bands grow at 255, 350 and 465 nm. TD-DFT calculations allow to assign the 465 nm band (λ_{calc} = 441 nm) to an $n-\pi^*$ transition and the 350 nm band (λ_{calc} = 320 nm) to a $\pi-\pi^*$ one, both being centered on the β molecular orbitals. The HOMO(α) → LUMO(α) transition is found at a slightly lower energy (λ_{calc} = 447 nm), being however associated with a very small oscillator strength (see Table 1) and thus hidden under the more intense n-π* visible absorption. It can be noticed that a weak absorption band also appears upon reduction in the 550–600 nm range, as evidenced by an isosbestic point (see inset in Fig. 1A). Finally the broad UV absorption band at 270 nm appears to be a complex mixture of various transitions. In summary, the anion radical spectrum shows mainly a hypsochromic shift of the n-π* transition, which corresponds with electronic repulsion between the single electron in the HOMO(α) and the one promoted to the LUMO(β).

The presence of the isosbestic points in the experimental electron absorption spectra and the good agreement with the calculated ones confirm the stability of the radical anion of ClTzOMe in the temperature interval for our experimental conditions. Although isosbestic again, its reoxidation led only to ca 85% recovery of the neutral parent compound (Fig. 1B). On the contrary the subsequent second and third redox cycles were completely reversible. We relate this discrepancy tentatively to product solubility or adsorption issues.

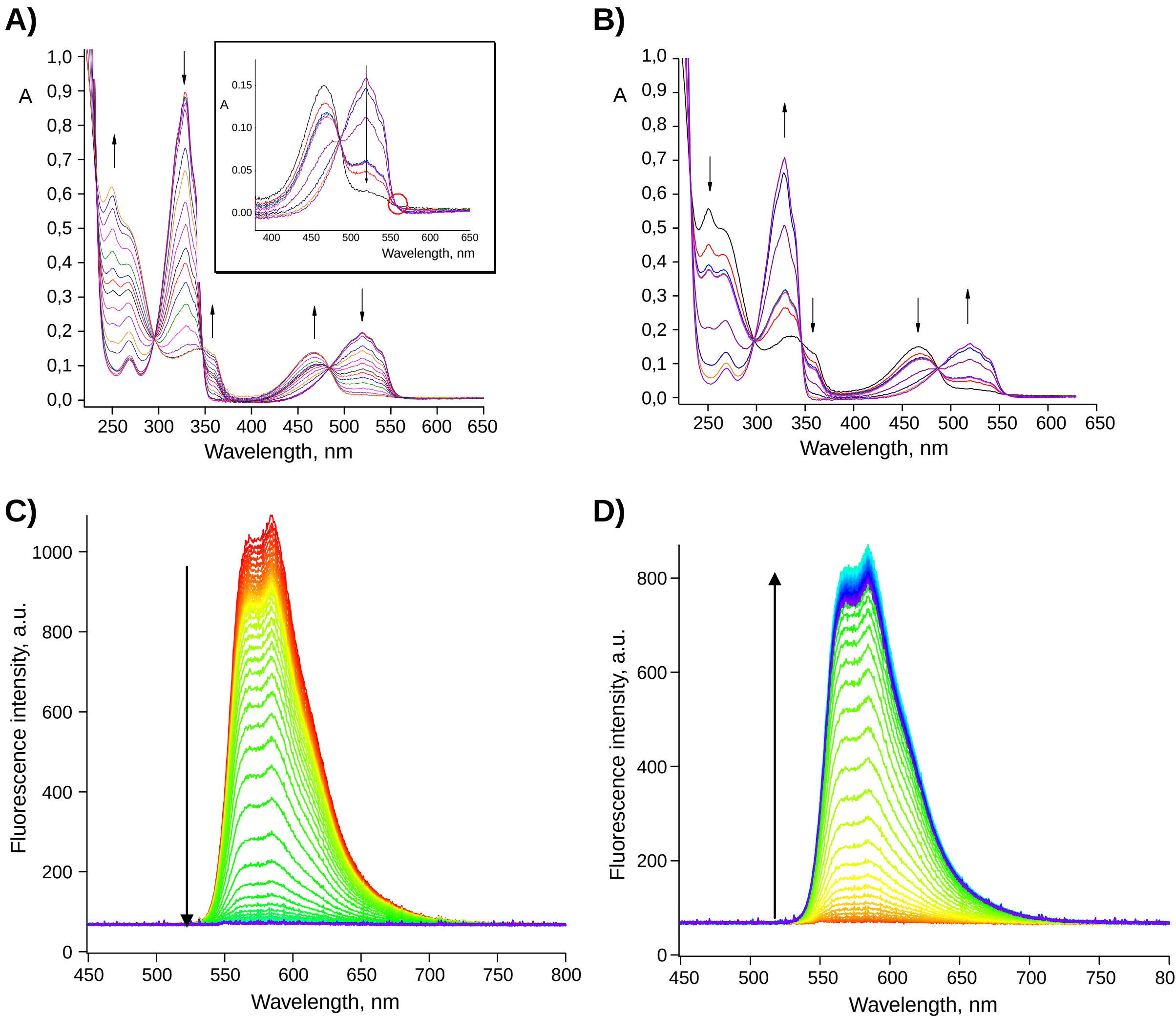

Fig. 1. Top : UV–vis spectroelectrochemistry in an OTTLE cell at T = 238 K in dichloromethane corresponding to: A) reduction of ClTzOMe into its anion radical (Inset represents the visible part of the spectrum for the reduction of ClTzOMe at T = 283 K); B) reoxidation of the anion radical into the neutral parent compound. Bottom : fluorescence spectroelectrochemistry of ClTzOMe in an OTTLE cell positioned under an epifluorescence microscope in dichloromethane. Spectra recorded in the order from red to blue : C) forward reduction step from 0 to −0.6 V during 60 s; D) backward reoxidation step.