

- [3] Grober M.S. Brittle stars bioluminescence functions as an aposematic signal to deter crustacean predators. *Anin. Behav.* 1988; **36**: 493–501.
- [4] Guilford T. & Cuthill I. Aposematism and bioluminescence. *Anim. Behav.* 1989; **37**: 339–341.
- [5] Leimar O., Enquist M. & Sillén-Tullberg B. Evolutionary stability of aposematic coloration and prey unprofitability – a theoretical analysis. *Am. Nat.* 1986; **128**: 469–490.
- [6] Mappes J., Marples N. & Endler J.A. The complex business of survival aposematism. *Tree* 2005; **20** (11): 598–603.

### First description of the luminous system of the velvet belly lantern shark *Etmopterus spinax* (Chondrichthyes: Etmopteridae)

Renwart M. & Mallefet J. (Marine Biology Laboratory, UCL, Belgium)

Catholic University of Louvain (UCL), 3, Place Croix du Sud, bt 4, Louvain-la-Neuve, 1348, Belgium

In the deep-sea, bioluminescence is more present in bony fishes (70%) than in cartilaginous fishes (6%)<sup>[1]</sup>. From literature, it is known that among sharks, many deep-sea species are able to produce light. Nevertheless, until recently, available data were anecdotic or mainly limited to morphological information. Recently a multidisciplinary research program (morphological, physiological, behavioural) was focused on the velvet belly lantern shark, *Etmopterus spinax*<sup>[2,3]</sup>. The aim of the present work was to document the nature of the luminous reaction involved in *E. spinax* luminescence.

Juveniles, adults and embryos of *E. spinax* were captured by long line lowered on the bottom of a fjord near Bergen (Norway).

Various tissues were collected and stored at –80°C before assays for luminous compounds.

Free coelenterazine and a specific luciferase-like activity have been detected in most tissues of the shark. In embryos, coelenterazine is present in the yolk sac. During embryogenesis, the yolk sac decreases in size and the coelenterazine is progressively absorbed by the growing embryo. Nevertheless, no increase of the luciferin concentration has been observed in the tissues of the embryos. At the end of the development, specific luciferase-like activity was detected in the embryo, indicating that they could be able to produce light before birth. This is in concordance with Claes & Mallefet (2) who observed a luminous embryo. In free-swimming specimens, coelenterazine has been found in all tissues tested, with the higher response in the photophores of new-borns (10–20 cm total length) (Fig 1). The presence of the luciferin in the digestive tract of the shark suggests an alimentary acquisition of this compound as it has been suggested for other fishes<sup>[4]</sup>. Luciferase-like activity has been detected in the different tissues with the higher response in the photophores of mature sharks (>30 cm TL) (Fig 2). Besides the somatic tissues, tests have been carried out on the gonads, for males and females independently. While coelenterazine concentration doesn't change in males, mature females show a decrease of luciferin, reaching a lower concentration than mature males. This decrease could reveal a maternal transfer of coelenterazine to embryo via the yolk sac. A similarly hypothesis was already suggested for teleost fishes<sup>[5]</sup>. Our results provide the first information on the luminous system of a shark: the presence of a luciferin/luciferase-like reaction, coelenterazine being the luciferin. Two mechanisms for coelenterazine acquisition are suggested (maternal transfer

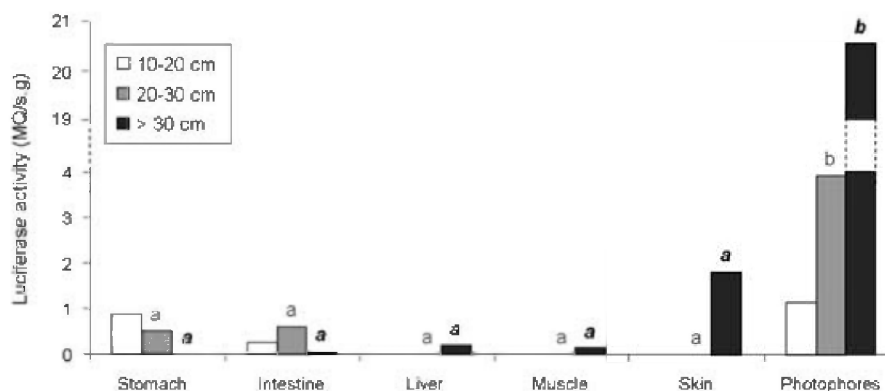


Figure 1. Coelenterazine concentration in the tissues, for each age classes: new-borns (10–20 cm TL); juveniles (20–30 cm TL) and matures (>30 cm TL).

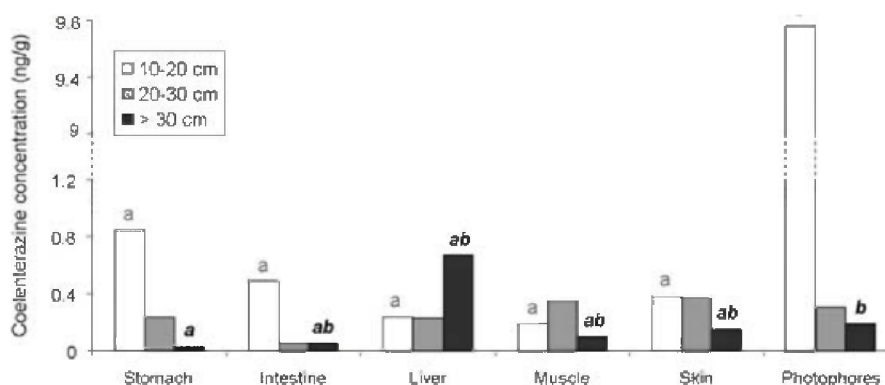


Figure 2. Luciferase activity in the tissues, for each age classes: new-borns (10–20 cm TL); juveniles (20–30 cm TL) and matures (>30 cm TL).

before birth, alimentary acquisition after birth). Work is in progress in order to determine which sources and forms of coelenterazine are used by the shark. We will also study coelenterazine fluxes in the shark and in its food chain. Complementarily, the characterisation of the luciferase will be undertaken.

## References

- [1] J.M. Claes, in *Bioluminescence in focus – A collection of illuminating essays*, (Ed. Meyer-Rochow), Research Signpost, India, **2009**, pp. 51–65.
- [2] Claes J.M. & Mallefet J. Early development of bioluminescence suggests camouflage by counter-illumination in the velvet belly lantern shark *Etmopterus spinax* (Squaloidea: Etmopteridae). *J. Fish Biol.* 2008; **73**: 1337–1350.
- [3] Claes J.M. & Mallefet J. Hormonal control of bioluminescence from lantern shark (*Etmopterus spinax*) photophores. *J. Exp. Biol.* 2009; **212**: 3684–3692.
- [4] Mallefet J. & Shimomura O. Presence of coelenterazine in mesopelagic fishes from the Strait of Messina. *Mar. Biol.* 1995; **124**: 381–385.
- [5] Rees J.F., Thompson E.M., Baguet F. & Tsuji F.I. Evidence for the utilization of coelenterazine as the luminescent substrate in *Argyrops leuciscus* photophores. *Mol. Mar. Biol. Biotechnol.* 1992; **1**: 219–225.

## Ru(bpy)<sub>3</sub><sup>2+</sup>-Pd nanoparticle-doped Carbon Composite Electrode as a Sensitive Solid State Electrochemiluminescence Sensor for the Detection of Amitriptyline

A. Safavi\*, F. Sedaghati

Department of Chemistry, College of Sciences, Shiraz University, Shiraz, 71454, Iran

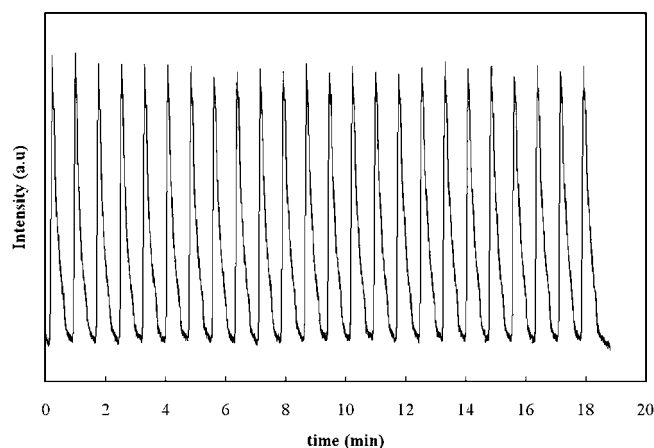
Nanotechnology Research Institute, Shiraz University, Shiraz, Iran

Tricyclic antidepressant drugs (TCAs) are one of the main groups of drugs for the treatment of psychiatric disorders such as depression, mainly endogenous major depressions. Amitriptyline hydrochloride is a tricyclic antidepressant showing sedative effects on anxious nervousness and on psychomotor nervousness. It also shows anticholinergic and antihistaminic effects.<sup>[1]</sup> Monitoring of this medicine is important for quality confidence in preparation and for achievement of optimum therapeutic concentrations, while minimizing the peril of toxicity.

Several methods were presented to determine TCAs, including radioimmunoassay, spectrofluorimetry, gas chromatography and high performance liquid chromatography (HPLC) using UV absorbance, chemiluminescence or electrochemical detection.<sup>[2–4]</sup>

However, these drugs do not absorb very well in the UV-visible region due to low molar absorptivities. Electrochemiluminescence (ECL) has been proven to be a powerful detection technique. The ECL signal may be significantly improved by immobilizing luminophores on electrode surfaces, forming solid state ECL detectors. Up to now, the Ru(bpy)<sub>3</sub><sup>2+</sup> complex exhibits the highest ECL efficiency, and has been extensively used in construction of solid state ECL sensors.<sup>[5]</sup> Various efforts to immobilize Ru(bpy)<sub>3</sub><sup>2+</sup> on an electrode surface have been made in order to fabricate an ECL sensor. The combination of metal nanoparticles and metal complexes leads to materials with interesting electrochemical and photonic properties.

In this report, Ru(bpy)<sub>3</sub><sup>2+</sup>-Pd nanoparticles doped carbon composite electrode was used for constructing a high performance solid state ECL nanosensor. In spite of the ability of



**Figure 1.** Consecutive scans of amitriptyline (2 μM) in phosphate buffer solution.

the metal nanoparticles to quench the ruthenium based emission, the ECL signal of this new nanocomposite was enhanced considerably.

The modified electrode was used for the ECL detection of amitriptyline and showed high sensitivity for the determination of this drug. High stability of the composite electrode was explored during 24 consecutive scans in phosphate buffer containing 2 μM amitriptyline (Fig. 1).

## References

- [1] J. Wang, M. Bonakdar, C. Morgan, Voltammetric measurement of tricyclic antidepressants following interfacial accumulation at carbon electrodes. *Anal. Chem.*, May 1986; **58**(6): 1024–1028. DOI: 10.1021/ac00297a009.
- [2] G.M. Greenway, S.J.L. Dolman, Analysis of tricyclic antidepressants using electrogenerated chemiluminescence. *Analyst* 1999; **124**: 759–762.
- [3] S. Ulrich, J. Materns, Solid-phase microextraction with capillary gas-liquid chromatography and nitrogen-phosphorus selective detection for the assay of antidepressant drugs in human plasma. *Journal of Chromatography B: Biomedical Sciences and Applications*. 1997 Aug 29; **696**(2): 217–234.
- [4] T.A. Ivandini, B.V. Sarada, A. Fujishima, Electrochemical detection of tricyclic antidepressant drugs by HPLC using highly boron-doped diamond electrodes. *J. Electroanal. Chem.* 2002 Mar 8; **521**(1–2): 117–126.
- [5] W. Miao, Electrogenerated Chemiluminescence and Its Biorelated Applications. *Chem. Rev.*, 2008; **108**(7): 2506–2553.

## Chemiluminescent screen-printed biochips for the simultaneous determination of four point-of-care relevant proteins

Françoise Bouteille, Benjamin P. Corgier, Agnès Degiuli, Loïc J. Blum and Christophe A. Marquette\*

Laboratoire de Génie Enzymatique et Biomoléculaire, Université Lyon 1 – CNRS 5246 ICBMS, Bât CPE, 43, bd du 11 novembre 1918, 69622 Villeurbanne, Cedex, France. Tel: +334 72 43 14 84, Fax: +334 72 44 79 70, christophe.marquette@univ-lyon1.fr

In the field of point-of-care (POC) analytical devices, low density microarrays (up to 10 parameters) are most of the time sufficient enough to get valuable information from the patient blood or