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Luminescence of *Leuco*-Thiazine Dyes

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Details of the novel luminescence of the *leuco* forms of the thiazine dyes, methylene blue and thionine, are reported, including their emission maxima, quantum yields and lifetimes of the luminescence. Other work shows that this luminescence is independent of reducing agent type and solution pH and is a common feature of most thiazine dyes.

KEY WORDS: Thiazine dyes; methylene blue; *Leuco-Methyle blue*; fluoresence.

INTRODUCTION

Thiazine dyes, such as thionine (Th) and methylene blue (MB) are widely studied and used. For example, as photosensitisers they are commonly employed in photogalvanic cells for solar to electrical energy conversion [1] and in photodynamic therapy for the treatment of cancer as a means of producing singlet oxygen [2,3]. They are also used as photodynamic anti-microbial agents in biological systems and materials, such as DNA [3–5] and are popular biological stains [6]. Finally, they are often used as a model test pollutant in semiconductor photocatalysis [7]. The structures and redox reactions relating MB and *leuco*-MB (LMB) and Th and *leuco*-Th (LTh) are illustrated in Scheme 1.

Thiazine dyes are also readily reduced to their colourless, *leuco* forms (eg. See scheme 1) by mild reducing agents, such as ascorbic acid [8] or sugars [9,10], such as glucose. Indeed, the redox indicator properties of these dyes form the basis of a well established, standard test for fresh milk [11]. These redox indicator properties also underpin a wide range of oxygen indicators, such as the Ageless Eye^{TM}[12], and the popular undergraduate "blue bottle" experiment [9,10]. Although the photochemistry of the thiazine dyes has been well investigated over the years, surprisingly, the photochemistry of their *leuco* forms has not. This paper details the previously unrecognized fluorescence of the *leuco* forms of the thiazine dyes, MB and thionine.

In a typical experiment, a nitrogen purged, pH 2 aqueous solution of MB (10^{-5} mol dm^{-3}) was reduced to LMB using freshly prepared zinc-amalgam. After reduction the solution was transferred to a fluorescence cell, whereupon its absorption, and uncorrected excitation and emission spectrum were recorded and the results are illustrated in Fig. 1. The adsorption spectrum of LMB illustrated in the main diagram in Fig. 1 is as reported by others [7,13] and exhibits 2 major bands at 314 nm and 256 nm, respectively. Excitation of LMB at either of these absorption bands reveals a blue luminescence with an emission maximum at 460 nm. The uncorrected excitation spectrum ($\lambda_{em} = 460$ nm) and emission spectrum ($\lambda_{excit} = 320$ nm) of LMB associated with this blue luminescence are illustrated in the insert diagram of Fig. 1. The excitation spectrum has a similar spectral profile to that of the absorption spectrum of LMB, indicating the emission is derived from an electronically-excited state of LMB. The emission spectrum of LMB peaks at 452 nm and has a minor peak at 360 nm due to Raman emission by the aqueous solvent. The quantum yield of fluorescence for LMB was determined to be 0.037, using quinine sulfate as a fluorescence secondary standard [14]. Single photon counting revealed the lifetime of the luminescence of LMB to be 2.35 ± 0.01 ns. Further work showed that LMB produced by a number of different routes, including using

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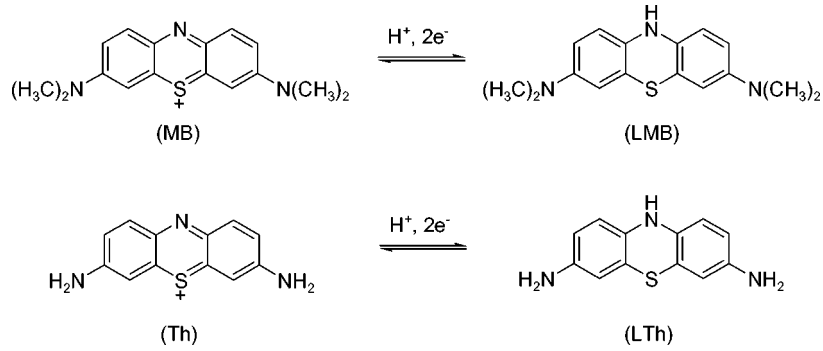

Scheme 1

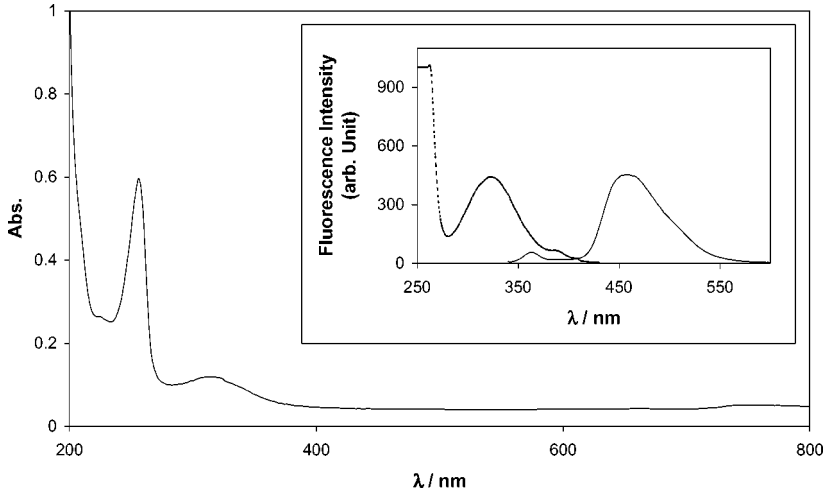

Fig. 1. UV/Visible absorption spectrum of an 10^{-5} mol dm^{-3} aqueous solution of *leuco*-methylene blue (LMB), at pH 2. The insert diagram illustrates the excitation spectrum (broken line, left hand side; $\lambda_{em} = 460$ nm) and emission spectrum (solid line, right hand side; $\lambda_{excit} = 320$ nm of the LMB solution.

chemical reducing agents such as ascorbic acid and sodium dithionite, exhibited the same characteristic luminescence as illustrated in Fig. 1.

At pH 2 LMB is slowly oxidized to MB by oxygen and it is possible to monitor this process via the decay of the luminescence due to LMB (452 nm) and the concomitant appearance of the luminescence due to MB at (682 nm) as a function of time using a wavelength of excitation of 256 nm. The results of this work are illustrated in Fig. 2 and highlight the expected inverse correlation between the luminescence from the two different species involved.

A similar set of experiments to those described above were conducted using the thiazine dye, thionine and its *leuco* form, and the results of this work, along with those for MB are summarized in Table I. From these results

Table I. Absorbance and Emission Characteristics of the Oxidized (Oxid) Dyes Methylene Blue and Thionine and Their Reduced (Red) *leuco* Forms

	Absorbance		Fluorescence					
	Oxid	Red	Oxidised form			Reduced form		
	λ_{max}/nm	λ_{max}/nm	λ_{max}/nm	Lifetime/ps	Quantum yield	λ_{max}/nm	Lifetime/ps	Quantum yield
Methylene blue	665, 292, 246	314, 256	682	358 ± 20	0.02 [4]	452	2368 ± 15	0.037
Thionine	600, 282	310, 262	610	320 ± 60 [15]	0.05	462	1158 ± 10	0.079

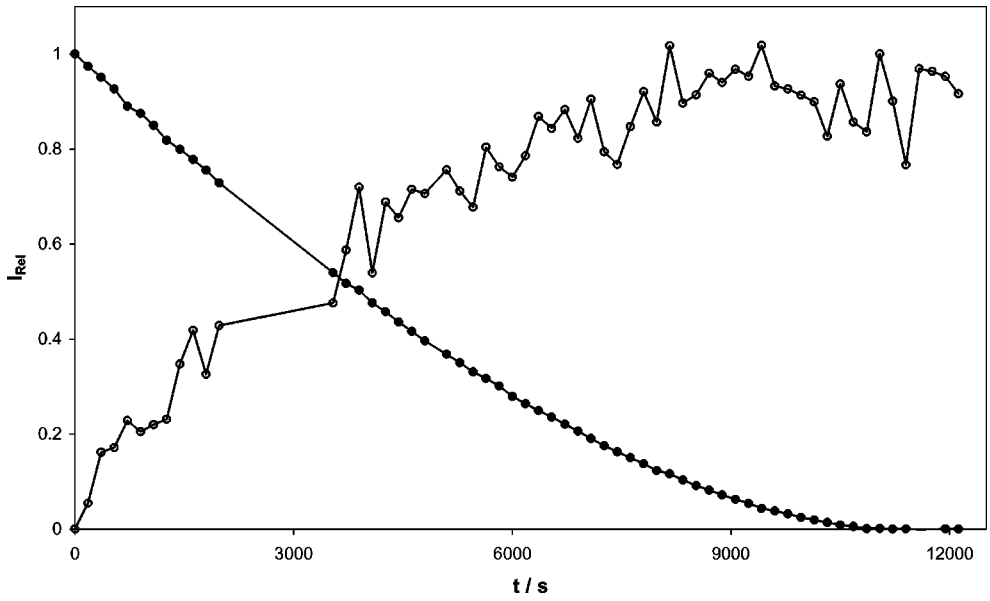

Fig. 2. Relative emission intensity versus time profiles recorded at: (i) 452 nm, $\lambda_{excit} = 256$ nm, (•), due to LMB and (ii) 682 nm, $\lambda_{excit} = 256$ nm, (○), due to MB after a nitrogen-purged solution of LMB (pH 2, 10^{-5} mol dm^{-3}) was opened up to air.

it can be seen that for the two thiazine dyes, MB and thionine, their *leuco* forms exhibit a stronger, relatively long-lived blue luminescence, compared to that of their oxidized forms. Further work on other thiazine dyes indicates that most thiazine dyes exhibit a similar luminescence in their *leuco* form.

To our knowledge, recognition and characterization of the luminescence of *leuco* thiazine dyes have never been reported previously. Such a feature will prove invaluable in aiding the identification and monitoring the of concentration of most *leuco* thiazine dyes.

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