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Fluorescence characteristics of sodium fluorescein–rose bengal ophthalmic solution mixtures

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ABSTRACT

Purpose: To assess fluorescence emission properties of sodium fluorescein–rose bengal mixtures in buffered aqueous solution.
Methods: Solutions of sodium fluorescein (NaFl) or rose bengal (RB) or mixtures of these two chemicals were prepared over a range of dilutions in 1% NaCl with 10 mM phosphate buffer to give a pH of 7.5 at room temperature. Absorbance and fluorescence spectra were recorded in 10 mm path length cuvettes.
Results: The fluorescence emission from NaFl extends between 480 and nearly 600 nm, a spectral range that is also covered by the absorbance of RB (between 500 and 580 nm). With very dilute solutions of NaFl (less than 0.002%), an apparent total quenching of its fluorescence can occur in the presence of 0.01% RB, with a proportionate decrease at lower concentrations of RB.
Conclusions: The presence of RB in an aqueous solution of NaFl at physiological pH appears to act in a similar way to a barrier filter, resulting in the quenching of the measurable fluorescence from NaFl. It remains to be established how substantial or significant such an effect might be if a mixture of NaFl and RB as used as part of the examination of the external eye.
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1. Introduction

Fluorescein is an orange coloured chemical that has been used for many years as an aid to diagnosing abnormalities of the ocular surface and tear film [1–7]. On illumination with blue light, it shows a characteristic yellow–green fluorescence emission that can then be viewed at the ocular surface with a slit-lamp suitably fitted with such an light source [8]. From assessments of various instruments, the actual wavelength range of the blue light source is broad and perhaps more likely to be at shorter (about 450 nm) rather than longer (about 490 nm) wavelengths [8]. Notwithstanding, in many assessments of the ocular surface with sodium fluorescein (NaFl), it can be broadly assumed that experienced practitioners learn to use just the right amount of topically-presented NaFl to obtain an optimum fluorescence that will allow them to discern gross or subtle alterations in the ocular surface and/or tear film. This is important, since a phenomenon of fluorescence quenching, sometimes referred to as internal- or self-quenching, occurs with NaFl solutions, i.e. above a certain concentration, the apparent fluorescence of aqueous solutions of NaFl will decrease [3,9–12]. The phenomenon is broadly due to overlap between the emission and excitation wavelengths, but it is also possible that there is an apparent spectral shift of the emitted light to longer wavelengths, depending on the wavelength used to elicit the fluorescence [7,11,13]. Regardless of whether or not there is a measurable spectral shift in internally quenched NaFl solutions, the phenomena can be broadly attributed to the fluorescence emission to the detector or viewing system being blocked by stronger internal absorption of the emitted wavelengths by very high concentrations of NaFl. This is because the absorption spectra are relatively broad (i.e. between 450 and 530 nm) and can be close to, or can overlap the emission maximum (that can be between 505 and 535 nm depending on the concentration of NaFl and the pH of the solutions) [7,11].

Another chemical that has been widely used as an ophthalmic diagnostic agent is rose bengal (RB), with its principal use being considered to be as a 'vital' stain to detect desiccated or damaged cells on the ocular surface [14]. This crimson-coloured chemical is a synthetic fluorescein derivative with the principal component probably being 4,5,6,7-tetrachloro-2′,4′,5′,7′-tetraiodo fluorescein [15]. In contrast to fluorescein, RB has a peak spectral absorption at slightly longer wavelengths to NaFl (i.e. around 550 nm) [16,17], but also secondary absorption that theoretically extends into the spectral range of fluorescein fluorescence Rose bengal itself only exhibits weak fluorescence, typically at longer wavelengths to the absorption maxima at 550 nm.

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Overall, in the clinical use of NaFl or RB to assess the ocular surface, it is likely that either chemical is presented to the eye separately, either in the form of preserved or preservative-free eyedrops or from ophthalmic wafers. In such a scenario, providing time is taken to allow for each chemical to be washed from the ocular surface, no interaction would be expected. However, one approach to assessment of ocular surface staining has been the use of a mixture of the two chemicals, e.g. NaFl 2% or 1% with RB 1% in an aqueous solution [18–22].

With the combined use of NaFl and RB in the clinical setting, no consideration seems to have been given to possible spectral interactions. The present study was prompted by observations that after RB eyedrops or strips had been used to stain the marginal conjunctiva (Marx's line) [23], subsequent slit-lamp evaluation with NaFl showed notably dim fluorescence from the fluorescein at the ocular surface and tear film. This sometimes occurred even 10 or 15 min after the RB staining had been undertaken prompting consideration of just whether or not RB could substantially quench the fluorescence of NaFl.

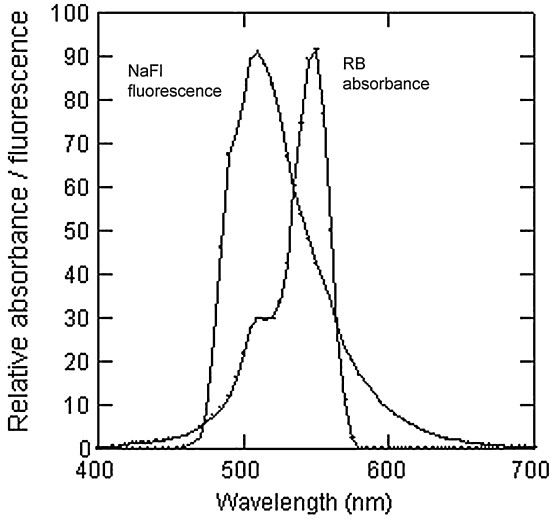

Fig. 1. Spectral data to illustrate how the fluorescence emission from sodium fluorescein (NaFl) overlaps the spectral absorption of rose bengal (RB). The spectra are in relative units only.

2. Methods

2.1. Chemicals

The source of sodium fluorescein (NaFl) or rose bengal (RB) were commercially available preservative-free ophthalmic solutions in the form of Minims® Fluorescein 2% or Minims® Rose Bengal 1% (both from Bausch & Lomb Surgical, Romford, UK). Various solutions were prepared by dilution of very small aliquots (10 or 20 μL into 2 mL) of the contents of the Minims® into reagent grade 1% NaCl (Sigma) with added 10 mM phosphate buffer (NaH_2PO_4–Na_2HPO_4 mixtures). A series of 1:1 dilutions were then made, with sets of repeat measurements being made in different batches of solutions. The buffered saline solutions were prepared in high purity water for infusion (Baxter Healthcare Ltd., Norfolk, UK). The pH of the solutions was verified using a temperature-compensated (for 20–22 °C) glass electrode prior to addition of the NaFl or RB.

2.2. Spectral determinations

Visible absorption spectra were recorded at room temperature (20–22 °C) using 10 mm path length cuvettes in a Beckman DU-640 recording spectrophotometer (Beckman Instruments, Palo Alto, CA) while fluorescence spectra were measured using a Gilford Fluoro-IV recording spectrofluorimeter (Corning Labs, Oberlin, OH), both at a nominal resolution (wavelength step) of 1 nm (with a stated repeatability of 1 nm given in the manual), i.e. these are the settings provided on the instruments. The recording spectrophotometer has a software programme (TRACE) that allows for the absorbance at any particular wavelength to be manually read after the set of spectra were obtained, while the fluorescence data were obtained by re-running the spectra at a low scan rate to permit manual recording of actual emission intensities at each wavelength. The location of any peaks were noted, but the spectra are presented here at a lower resolution of 5 nm.

3. Results

3.1. Overall character of absorbance and fluorescence spectra of sodium fluorescein and rose bengal

Dilute to very dilute (i.e. <0.002%) aqueous solutions of NaFl show a distinctive peak absorbance at 490 nm (not shown, but see Fig. 1 [7]). In addition, at neutral to slightly alkaline pH values, there is a slight shoulder on the spectral absorbance between 440 and 460 nm. Illumination of very dilute solutions of NaFl with blue light of 490 nm results in a very pronounced yellow–green fluorescence with the peak emission being close to 505–510 nm (see Fig. 1). If the blue light includes shorter wavelengths then the total measured fluorescence should still be substantial. However, if the blue light was restricted to shorter wavelengths (e.g. between 440 and 460 nm) then this net fluorescence will be much less.

RB solutions show a similarly complex absorbance spectra to NaFl but now with a dominant peak at 550 nm and more distinctive shoulder at shorter wavelengths close to 500 nm (see Fig. 1). RB solutions can emit very weak fluorescence on excitation at 490 nm but with excitation between 440 and 465 nm, the fluorescence increases substantially with the peak emission being between 570 and 580 nm (not shown, but see Fig. 2).

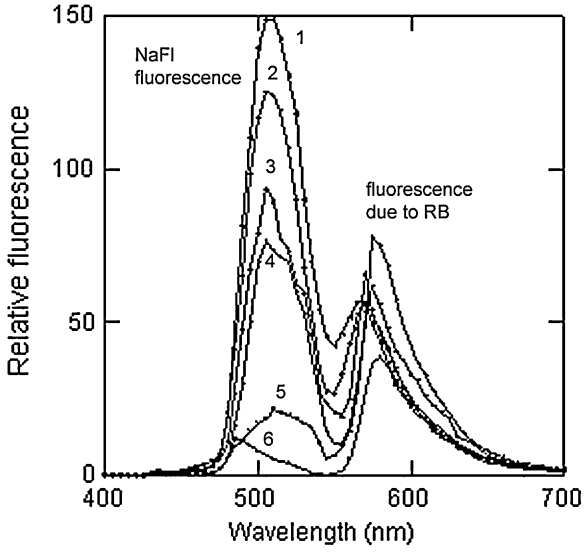

Fig. 2. Fluorescence emission spectra of sodium fluorescein (with emission peak centred at between 505 and 515 nm) in the presence of increasing concentrations of rose bengal (from top trace 1 to bottom trace 6, where the peak in this region is now close to 490 nm). The fluorescence emission at longer wavelengths between 560 nm and 585 are presumed to be derived from rose bengal. The fluorescence intensity is in arbitrary units. Phosphate buffered saline at pH 7.5.

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