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Inner filter effects and other traps in quantitative spectrofluorimetric measurements: Origins and methods of correction ☆

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HIGHLIGHTS

- Nonlinear instrument-dependent relationships complicate the use of spectrofluorimetry to measure concentrations.
- The instrumental factors affecting the luminescence intensity observed in solution at fixed wavelength are discussed.
- A simple data correction method to obtain emission intensity values proportional to luminophore concentrations is proposed.

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ABSTRACT

Spectrofluorimetry is an analytical technique endowed with excellent versatility and high sensitivity. The low cost, handiness of use, and compact size of modern spectrofluorimeters has fostered their widespread availability in chemical laboratories. However, the utilization of spectrofluorimetry to determine concentrations – an essential task to investigate intermolecular association phenomena – is complicated by the non-linear instrument-dependent relationship between the concentration of the luminescent analyte and the detected emission signal, as well as by the spectrophotometric characteristics of the sample. Here we discuss the instrumental factors affecting the luminescence intensity observed in solution experiments with fixed excitation and emission wavelengths, and we propose a simple data correction method that converts the measured intensity value into a quantity which is linearly proportional to the concentration of the luminophore of interest. Two examples illustrating the method and its application for the study of self-assembly processes, taken from our research, will also be presented.

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Introduction

The effect of light on matter has long been attracting the interest of researchers from many different fields, both on fundamental and applied studies, spanning from investigations on processes involved in the origin of life on earth, to design new solutions useful for everyday life such as material and environmental sciences, energy production, medical diagnosis and therapeutics, and data storage [1,2]. For this reason, photochemistry is still undergoing a tremendous development [3]. In particular, the interest is shifting from purely molecular systems to supramolecular architectures [4,5] and nanostructures [5–8], where intermolecular interactions can result in novel photochemical and photophysical properties. Such architectures are in fact an ideal platform to couple elementary processes (light absorption and emission, energy and electron transfer) to give rise to more complex ones (e.g., directional excitation energy migration or multi-electron photoinjection) in order to design nanosized functional photochemical devices [1–7].

In this context, the phenomenon of luminescence – that is, the radiative deactivation of an electronically excited state – and the parameters related to it, such as intensity, anisotropy, emission and excitation spectra, and excited state lifetime, can be used to investigate the behaviour of the species of interest as well as to probe its environment [9]. In general, luminescence-based techniques offer a very high sensitivity, with a linear response in a wide concentration range even in complex matrices, so that, in special conditions, even single molecule detection is possible [10]. Another advantage of luminescence-based techniques is related to their versatility, which derives from the wide number of variables that can be tuned and coupled to get the sought information, allowing to tackle complex analytical problems. A first and very interesting element of versatility is given by the origin of the luminescence process itself. In this article we will mainly deal with photoluminescence (i.e., luminescence arising from an excited state created

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by the absorption of light), that is by far the most common methodology among the different luminescence techniques. It is however important to underline that (electro-)chemiluminescence (luminescence arising from an excited state generated by an (electro-)chemical reaction) [11] and thermochemiluminescence (in which the excited state is generated by the thermolysis of suitable molecules) [12] can offer even higher sensitivity and ease of use.

Unfortunately, besides all these valuable features, luminescence measurements have also some limitations. In fact, they are not always as easy as they could seem at a first glance [9,13–16], since they can often hide subtle artefacts. Furthermore, while the output signal produced by a spectrophotometer represents a physical quantity (the absorbance) that can be expressed in an absolute scale, the output produced by a spectrofluorimeter is related to the total luminescence intensity (i.e., to the number of emitted photons) through a number of instrumental factors (intensity of the exciting source, instrument optics, signal amplification) and to the sample characteristics. For these reasons, the observed intensity can be related to sample concentration only if corrected in order to take into account all these factors. We wish to stress that luminescence intensity measurements carried out with standard spectrofluorimeters are never absolute quantities, and the intensity values must be expressed in a relative scale even after corrections. As a consequence, the number given by the instrument has no direct physical meaning: only the ratios between values measured under the same experimental conditions can have a significance to analysts working with other equipments and in different conditions.

The relatively low cost, handiness of use and compact size of modern spectrofluorimeters has fostered their widespread availability in chemical laboratories. If users are not aware of the above mentioned facts, however, substantial mistakes in data interpretation can be made, leading to wrong conclusions. This article is a refinement of our prior papers on the same topic [17–19], enriched with our more recent experience and tailored for the scope of this journal. Our aim is to help readers understanding the problems related to luminescence measurements in solution and avoiding any possible misinterpretation.

Scope and limitations

In this article we will deal with quantitative steady-state luminescence intensity determination in solution, at a fixed emission wavelength, using a commercial spectrofluorimeter with right-angle excitation (perpendicular geometry). These kinds of measurements represent a commonplace in spectrofluorimetry and are particularly important in analyte detection, titrations, quenching and sensitization experiments, photoreaction and photoluminescence quantum yield determination, and whenever a luminescence signal is used to monitor a chemical process.

The determination of the "corrected" emission intensity of a luminophore from the luminescence values measured in an experiment implies the knowledge of (i) *the fraction of incident photons absorbed by the emitting species* and (ii) *the fraction of the actually emitted light that is detected by the instrument*. The corrections we are introducing take into account all the factors that affect the measured luminescence intensity, thus answering to the above questions. These factors are often collectively known as *inner filter effects* [15]. For the sake of clarity we prefer to refer to inner filter effects only in the cases in which co-absorption of the exciting light and/or re-absorption of the emitted light takes place. Therefore, the procedure that we propose to make corrections on observed luminescence intensities consists of two steps: (i) correction for the (non-linear) luminescence intensity response of the instrument vs. absorbance and (ii) correction for inner filter effects. For other problems such as resolution of the spectrum, phototube response, and interference from excitation and emission harmonics or spurious bands, we refer the interested readers to Refs. [18,19].

To better illustrate the proposed methodology, in the final part of the article we will describe its application to two case studies, taken from our research, regarding association processes of luminescent organic molecules, inorganic compounds, and nanocrystals.

Instrument calibration curve

Fig. 1 shows an experimental calibration curve obtained by plotting of the emission intensity of a luminescent species in solution as a function of its absorbance – and hence of its concentration. The graph shows that the luminescence intensity increases linearly upon increasing the absorbance only for very low absorbance values. For higher absorbances the emitted intensity increases in a nonlinear manner, until it reaches a maximum (usually around $A = 1$). Finally, for even higher absorbance values, the luminescence intensity decreases upon increasing the absorbance (i.e., the sample concentration). This means that, as we will discuss below, the observed luminescence intensity has no need of corrections only for very dilute solutions, where effects related to the absorption of the exciting and emitted light can be neglected [14]. This condition is often fulfilled in experiments; as a matter of fact measurements on high dilute samples represent one of the most important challenges for fluorimetric techniques in analytical chemistry. In many other instances, however, more concentrated solutions have to be examined. This is unavoidable if, for example, the brightness (see below) of the luminescent species is very low or an equilibrium is undesirably affected by dilution. Other problems may be present if the solution contains other chromophores absorbing at the excitation and/or emission wavelengths.

The shape of the calibration curve reported in Fig. 1 arises from the combination of two distinct factors: one of mathematical and one of "geometric" nature. The first factor can be easily understood considering that the emitted light is proportional to the number of the excited states present in solution, which are in turn

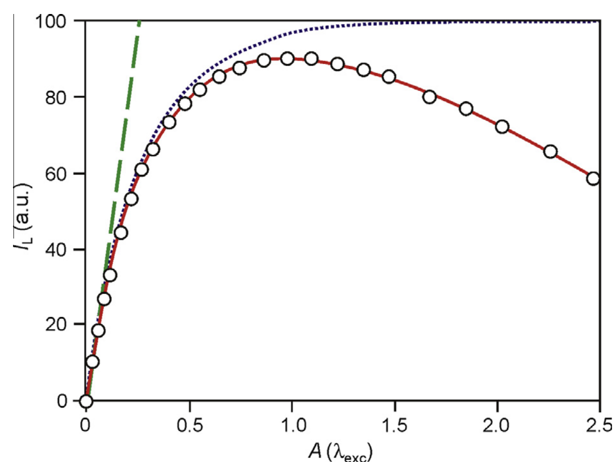

Fig. 1. Experimental calibration curve obtained by plotting the luminescence intensity observed for quinine sulfate solutions at different concentrations in H_2SO_4 0.5 M (empty circles, full line). Instrumental conditions: Perkin–Elmer LS-50 spectrofluorimeter, $\lambda_{exc} = 350$ nm, $\lambda_{em} = 450$ nm, bandpass 5 nm. The dotted line shows the luminescence intensity vs. absorbance relationship expected on the basis of the sole mathematical factor. The dashed line is the tangent to the experimental curve for $A(\lambda_{exc}) \to 0$, and evidences the linear relationship between luminescence intensity and absorbance for $A < 0.1$. Adapted with permission from Ref. [17].

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