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ORIGINAL PAPER

Polymer-and Glass-based Fluorescence Standards for the Near Infrared (NIR) Spectral Region

Christian Würth · Katrin Hoffmann · Thomas Behnke · Marius Ohnesorge · Ute Resch-Genger

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Abstract The widespread use and acceptance of fluorescence techniques especially in regulated areas like medical diagnostics is closely linked to standardization concepts that guarantee and improve the comparability and reliability of fluorescence measurements. At the core of such concepts are dependable fluorescence standards that are preferably certified. The ever rising interest in fluorescence measurements in the near-infrared (NIR) spectral region renders the availability of spectral and intensity standards for this wavelength region increasingly important. This encouraged us to develop approaches to solid NIR standards based upon dye-doped polymers and assess their application-relevant properties in comparison to metal ion-doped glasses. The overall goal is here to provide inexpensive, easily fabricated, and robust internal and external calibration tools for a broad variety of fluorescence instruments ranging e.g. from spectrofluorometers over fluorescence microscopes to miniaturized fluorescence sensors.

Keywords Fluorescence standard · PMMA · Photostability · Perylene · Rhodamine 800 · Dye-doped polymers · Glass · Laser dye

Introduction

Photoluminescence techniques, which yield emission and excitation spectra and analyte-specific quantities such as luminescence quantum yields, luminescence lifetimes, and emission anisotropies, are among the most widely used tools in the material and life sciences [1]. Limitations of these techniques are instrument-dependent contributions to otherwise analyte-specific fluorescence signals, challenges to measure absolute luminescence intensities [1–10], and general difficulties to accurately quantify from measurements of relative fluorescence intensities. The latter is closely related to the sensitivity of the spectroscopic properties of most chromophores (such as absorption and emission spectra, molar absorption coefficients, photoluminescence quantum yields, and luminescence lifetimes) to their microenvironment (in terms of temperature, viscosity, solvation, polarity, proticity, pH, ionic strength, presence of quenchers, and attachment to bio-or macromolecules). In addition, the measured fluorescence intensity correlates with the spectral irradiance reaching the sample and thus changes upon fluctuation of the excitation light intensity. This situation is further complicated by the existence of very few guidelines, recommendations, and technical notes for the characterization and performance validation of photoluminescence measuring instruments [11, 12] and the performance of measurements of relevant photoluminescence quantities [5, 13–16]. The only exceptions are here colorimetry or surface fluorescence [17, 18] and, in part, flow cytometry [19]. Also, only a comparably small, yet increasing number of recommendations on different types of chemical or chromophore-based fluorescence standards are available for the majority of photoluminescence measuring techniques [12–14, 16, 20–24].

The recognized need for concepts to improve the reliability of fluorescence measurements and quantitative fluorescence analysis led meanwhile to the development of new calibration tools for the ultraviolet (UV) and visible (vis) spectral region such as the certified liquid spectral standards F001 to F005 released by the Federal Institute for

C. Würth · K. Hoffmann · T. Behnke · M. Ohnesorge · U. Resch-Genger (✉)
BAM Federal Institute for Materials Research and Testing I.5,
Richard-Willstaetter-Str. 11,
12489 Berlin, Germany
e-mail: ute.resch@bam.de

Materials Research and Testing (BAM) in 2006 [25–27] and the certified glasses released by the National Institute of Standards and Technology (NIST) in 2007 [28–31]. However, the BAM standard F005 emits only up to 740 nm (fluorescence intensity ≤ 10% of the intensity at the emission maximum) and the only moderately emissive red NIST glass (photoluminescence quantum yield of transition metal ion-doped glasses about ≤ 0.1 [32]) up to 800 nm [28]. At present, there are almost no reliable standards available for the near-infrared (NIR) spectral region despite of the ever increasing interest in measurements of NIR fluorescence e.g. in the life sciences like *in vivo* fluorescence imaging in the first diagnostic window between 650 nm and 900 nm. Moreover, the trends towards multifunctional standards e.g. for the comparability of fluorescence measurements across different types of instruments, multiplexed detection [33, 34], and the miniaturization of fluorescence instruments require standards that can be easily adapted to different formats, i.e., measurement geometries, sizes and shapes, display preferably several emission bands, and can be even integrated into fluorescence instruments. Especially for miniaturized point of care measuring systems, which are often used in different environments and under different measurement conditions, such internal instrument validation standards are essential to ensure proper functioning of the device. Moreover, such measurement systems are only approved by regulatory agencies like the Food and Drug Administration (FDA) if reliable and evaluated standards for the regular instrument performance validation (IPV) are available or can be provided by the instrument manufacturer [35].

These trends encouraged us to develop strategies for the design of new instrument characterization and validation standards for the vis and NIR spectral region. Although liquid standards can be used in a broad variety of formats [16, 35], their longterm stability is typically limited, especially for NIR fluorophores, rendering them frequently unsuited as internal fluorescence standards for the integration into fluorescence instruments. Solid standards like the broad band and narrow band-emitting metal-ion doped glasses from NIST and BAM [5, 16, 28–31, 35, 36] are principally very attractive alternatives here, yet their fabrication requires special expertises. This also renders these materials comparatively expensive. This encouraged us to assess simple and inexpensive procedures for the preparation of polymer-based fluorescence standards containing a single dye, i.e., a vis or a NIR emitter, and mixtures of two fluorophores absorbing and emitting in the vis and NIR spectral region. The latter generizable approach offers access to materials with a tunable Stokes shift that can be excited at a comparably short wavelength, i.e., in the vis, yet emit in the NIR [37, 38]. Such materials can be exploited e.g. for referencing of the (analyte-sensitive) signal of a sensor system in the vis spectral region to the fluorescence output of an (analyte-insensitive) NIR-emitting standard using a single excitation wavelength to correct for fluctuations of the excitation light source, thereby extending the concept of dual emissive fluorescent probes and signal ratioing from fluorescence sensors [39, 40] to fluorescence standards. Moreover, signal ratioing with standards displaying two or more distinct emission bands can be advantageous for instrument performance validation (IPV). In contrast to conventional IPV standards like the sealed water cuvette used for the so-called Raman test [41, 42], that measures the performance of the excitation and emission channel simultaneously, the signal ratioing standards presented here provide a tool for the separate control of both channels. The application-relevant features of these new polymer-based standards like lateral homogeneity, longterm photostability, and emission anisotropy were investigated in detail and compared to the corresponding properties of glass-based broad band emitters and narrow band multi-emitters [5, 36] thereby underlining the potential and limitations of each type of reference material. All the materials studied here were chosen with respect to the ease of use in different formats and low cost of material production.

Experimental

Materials

Azo-bis-(isobutyronitrile) (AIBN) and methyl methacrylate (MMA) (purity 99%) were purchased from Sigma Aldrich (Germany). The perylene dye Lumogen F305 (F305) was purchased from BTC Europe and rhodamine 800 (Rh800) as well as rhodamine B (RhB) were obtained from Lambda Physik GmbH, Germany. All the dyes were used as received without further purification. Polymerization of the dye-monomer-initiator cocktails was performed in 10 mm×10 mm quartz cuvettes. Metal ion-doped glasses were generously provided by Schott AG [5, 36]. The cuvette-shaped rhodamine B-doped PMMA standard was purchased from Starna GmbH.

Methods

Preparation

The dye-doped polymers were prepared via polymerization of a MMA-dye cocktail. Prior to MMA use, the routinely added inhibitor (monomethyl ether hydroquinone) was removed with 1 M NaOH and water. The initiator AIBN and the dyes Rh800 and F305 were dissolved in MMA. The dye concentrations used were

between 1.6×10^{-5}M and 3×10^{-5}M with concentration ratios of Rh800 to F305 varying from 4.7 to 12.5, respectively. The amount of AIBN was varied from 0.1w % to 1w%. The dye-containing MMA solutions were transferred to quartz cuvettes for measurements of absorption and emission spectra and were subsequently polymerized during 12 h in a tempered (323 K) silicon bath. After the polymerization, the cuvette-shaped dye-doped polymers were heated up twice to 343 K for 23 h to remove nonreacted AIBN. This also enabled screening of the thermal stability of these materials. For the spectroscopic studies, blank or so-called reference samples were prepared using the same polymerization procedure as applied for the dye-doped polymeric systems, yet no fluorophores were added.

Spectroscopy

All the absorption and fluorescence measurements were performed at T=298 K ± 1 K in 10 mm cuvettes (unless otherwise stated). Absorption spectra were recorded on a Cary 5000 spectrometer. The accuracy of the intensity and the wavelength scale of this instrument was controlled using certified absorption standards from Hellma GmbH. Fluorescence emission spectra were measured with a previously described Spectronics Instruments 8100 spectrofluorometer in a 0/90° measurement geometry [25]. The calibration (traceable to radiometric scales) of the fluorometer SLM 8100 has been recently described [25, 26]. For typical emission measurements, the Glan Thompson polarizers placed in the excitation and the emission channel of the fluorometer 8100 were set to 0° and 54.7°, respectively. Under these so-called magic angle conditions, that are recommended for the accurate measurement of the fluorescence of non-isotropic emitters like most large NIR chromophores, the detected emission intensities are independent of the dye´s emission anisotropy (r) [43].

The emission anisotropy r (see equation 1), that represents the degree of polarization of the emitted light by a sample,

$$r = \frac{I_p(\lambda) - I_s(\lambda)}{I_p(\lambda) + 2I_s(\lambda)} \quad (1)$$

was determined from blank-and spectrally corrected emission spectra measured with the excitation polarizer set to 0° and the emission polarizer set to 0° (parallel polarizers; $I_p(\lambda)$) and 90° (perpendicular polarizers; $I_s(\lambda)$).

To correct for background signals, dye-free reference samples were used. If not otherwise stated, all the blank-corrected fluorescence emission spectra presented were corrected for the wavelength-and polarization-dependent spectral response of the detection system.

Fluorescence excitation spectra and fluorescence measurements for the longterm stability studies were performed with the fluorometer LS50B from Perkin Elmer. We did not attempt to correct the excitation spectra for the wavelength- and polarization-dependence of the spectral irradiance reaching the sample. For the longterm stability studies, the measurement conditions and settings were kept constant and the samples were stored at room temperature.

Fluorescence mapping

Fluorescence mappings were recorded with a x,y-translation stage from Linos (x.act XY 50-2 ST) using a fiber-coupled Ocean Optics CCD spectrometer (QE65000) in conjunction with diffractive optical elements as detection system. A monochromatic fiber-coupled light source (Xe-Lamp coupled with a single monochromator) and diffractive optics were employed as excitation channel. The angle between the optical axes of the excitation and the detection channel was 90°. We chose spot sizes of 1 mm. The measurement spots were adjusted with a dichroic mirror set to 45° relative to the optical axes of the detection and the emission channel. Prior to mapping, the cuvette-shaped (x=10 mm, y=10 mm) dye-doped polymer blocks were cut into slices of 1 mm and 4 mm along the z-direction and polished to eliminate influences from surface textures. For fluorescence mappings along the x- and y-direction of a slice, we used a measurement grid of 81 points. At each point, a fluorescence spectrum was recorded.

Photostability studies

Photostability studies were carried out with a 450 W Xe-Lamp combined with a single monochromator. Illumination was performed with an excitation wavelength of 590 nm using spectral irradiances of up to 0.15 mW/mm^2. The spectral irradiance at the sample position was measured with a calibrated silicon detector placed inside an integrating sphere. During illumination, the samples of 1 mm thickness were placed in a holder with an aperture. The use of a defined aperture guarantees the illumination of sample areas with homogeneous optical properties, which were identified prior to photostability studies by fluorescence mapping (see "Fluorescence mapping"). Care was taken to guarantee homogenous illumination (spots with uniform intensity). During illumination, the time dependent transmittance was recorded with a detector, located behind the sample. Before and after each illumination, the absorption spectra of the homogeneously illuminated areas of the polymeric samples were measured with the Cary 5000 spectrometer. To account for fluctuations of the excitation light intensity or spectral irradiance reaching the sample, a reference detector was implemented into the setup.

Results and discussion

Single and double fluorophore-doped polymers emitting in the NIR

The broad use of instrument validation standards is directly linked to straightforward methods for the reproducible and inexpensive fabrication of suitable reference materials. This encouraged us to investigate the potential of a simple and flexible polymerization procedure for the preparation of single-and two-component instrument validation standards for the vis and NIR spectral region.

We chose rhodamine 800 (Rh800) and the vis-emitting perylene dye Lumogen F305 (F305) as fluorophores due to their comparatively high fluorescence quantum yields (Φ_f) and assumed high stability. F305 has a Φ_f value near 100% [44, 45] and has been recommended for a variety of applications that require a very high dye stability such as electroluminescent devices [46–48] and solar concentrators [44, 49]. Rh800 is a laser dye and one of the very few xanthene chromophores that displays an emission above 650 nm [50–53]. The absorption (left) and emission (right) spectra of F305 and Rh800 in PMMA are shown in Fig. 1. Figure 2 summarizes the absorption spectra (left), fluorescence excitation spectra (left, recorded at two different emission wavelengths) and the fluorescence emission spectra (right, measured at different excitation wavelengths) of an exemplary chosen polymeric sample containing a typical mixture of both fluorophores. As follows from Fig. 2 (left panel), the excitation spectrum of the two component-system at 640 nm is rather similar to the absorption spectrum of F305, whereas the excitation spectrum at 760 nm is dominated by Rh800 with minor contributions from F305 (e.g. shoulder at ca. 570 nm). The emission above 750 nm (see also wavelength region D2, right panel of Fig. 2) can be excited with wavelengths between 400 nm and 750 nm. As shown in the right panel of Fig. 2, the emission spectrum of the two component system comprises several bands, the intensities and intensity ratios of which can be elegantly controlled by excitation wavelength as well as concentration ratio of the two fluorophores. This follows immediately from a comparison of the quotient (Q= D1/D2) of the integral emission intensities in the wavelength regions D1 and D2 as a function of excitation wavelength (Q_{410nm}=0.36, Q_{440nm}=1.4, Q_{580nm}=0.68, Q_{625nm}=0.09). Principally, these wavelength regions could be e.g. used for signal ratioing-based monitoring of longterm instrument performance during IPV.

Optimization of polymerization conditions

A general difficulty for the simultaneous incorporation of two chromophores into a polymeric matrix via a photochemically or thermally initiated polymerization can arise from the different stabilities of these dyes. This can be especially critical for combinations of vis and NIR chromophores as the latter often suffer from considerably reduced thermal and photochemical stabilities. Because of the well known and likewise observed high stability of F305 [44], we focused here on the optimization of the polymerization conditions for the more susceptible NIR dye Rh800. Aiming at the minimization of dye loss during polymerization, this reaction was performed with three different initiator concentrations (AIBN 0.1w%, 0.5w% and 1w%) and dye concentrations varying between 1.6×10^{-5}M and 3×10^{-5}M with ratios of 4.7 to 12.5 of Rh800 and F305, respectively. The loss in Rh800 concentration was determined from the change in absorbance at 685 nm relative to the corresponding absorbance of the dye dissolved in MMA, thereby assuming identical molar absorption coefficients of the fluorophore in MMA and PMMA.

As summarized in the left panel of Fig. 3, the MMA solutions with 0.1w% AIBN reveal the strongest decrease

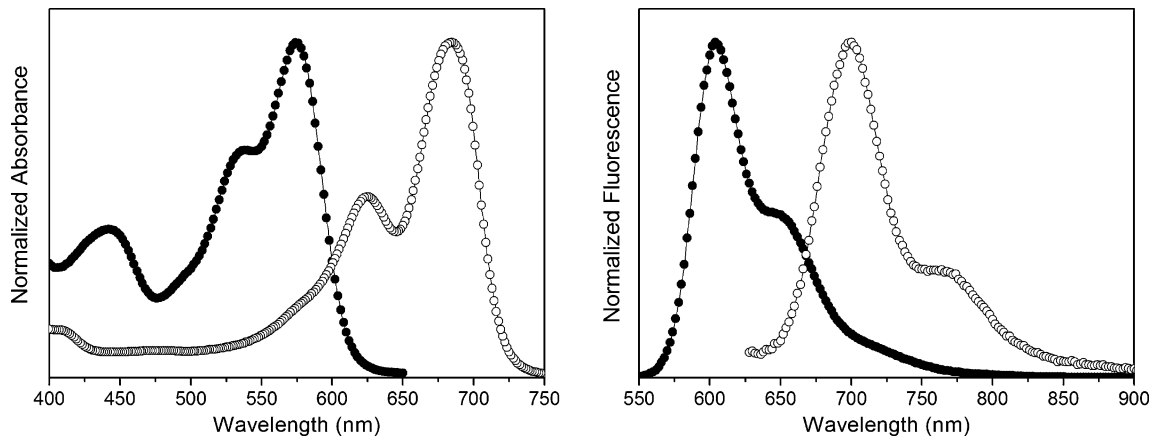

Fig. 1 Absorption (*left*) and fluorescence emission spectra (*right*) of PMMA, doped with Lumogen F305 (*solid symbols*) and rhodamine 800 (*open symbols*)

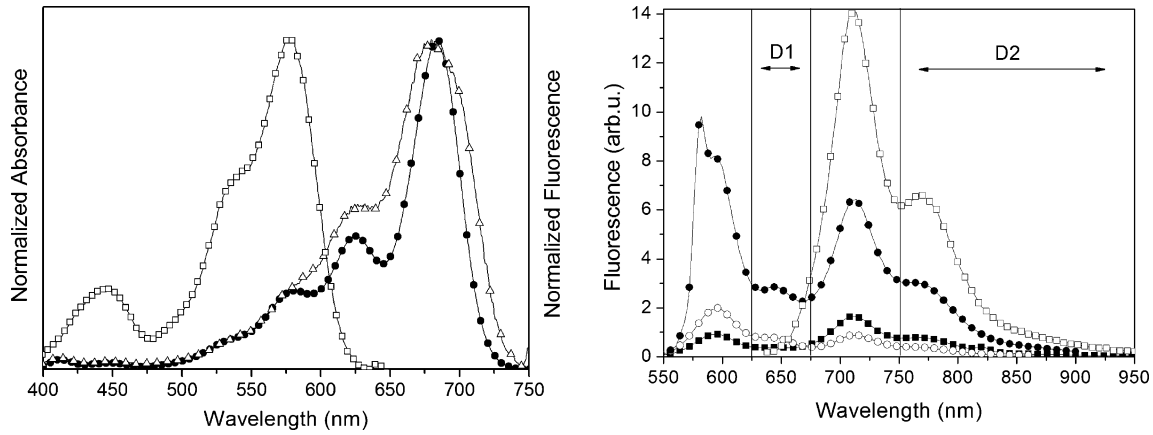

Fig. 2 Left: Absorption (*solid circles*) as well as uncorrected fluorescence excitation spectra recorded at two different emission wavelengths (λ_{em}=640 nm: open squares, λ_{em}=760 nm: *open triangles*) of a dual doped (F305, Rh800) polymer. Right: Corrected emission spectra measured at different excitation wavelengths (λ_{ex}=410 nm: *solid squares*, λ_{ex}=440 nm: open circles, λ_{ex}=580 nm: *solid circles*, and λ_{ex}=625 nm: *open squares*). D1 and D2 indicate the wavelength regions used for the homogeneity studies

in absorbance of up to 35% while the solutions polymerized in the presence of 1w% AIBN show a mean decrease of only about 6%, independent of dye concentration. The right panel of Fig. 3 illustrates the influence of heating (annealing at temperatures of up to 343 K for 23 h). In contrast to the polymerization performed at 323 K, the samples prepared in the presence of 0.1w% and 1w% AIBN display a diminished absorbance with increasing dye concentration. Appropriate polymerization conditions have been found with 0.5w% AIBN for dye concentrations of 1.6×10^{-5}M and 3×10^{-5}M with a mean decrease of 20%.

Dye-doped polymers as potential fluorescence standards for the NIR

General requirements on instrument characterization and validation standards are an excellent spatial homogeneity of the application-relevant spectroscopic properties suitable for the instrument to be characterized, a sufficient thermal and photochemical stability and a problem-adapted emission anisotropy [5, 16, 25, 27, 35, 54]. For example, for certain applications like the determination of the spectral responsivity of the emission channel, a small fluorescence anisotropy (r < 0.05) of the standard is strongly recommended to reduce polarization effects [25, 26].

Accordingly, we studied these key parameters for the investigated single and dual dye-doped polymeric systems in comparison to our "golden standards", i.e., broad band- and narrow band-emitting glasses emitting in the vis and NIR [35, 36]. Figure 4 displays the emission spectrum of a glass doped with a mixture of rare earth ions, displaying multiple narrow emission lines (multi-emitter (ME) glass) that is suitable as day-to-day intensity, signal ratioing, and wavelength standard, as well as the broad and unstructured emission spectrum of a Cr^{3+}-doped glass, suggested as a potential emission standard for the spectral region of about 700 nm to 1050 nm [16, 35].

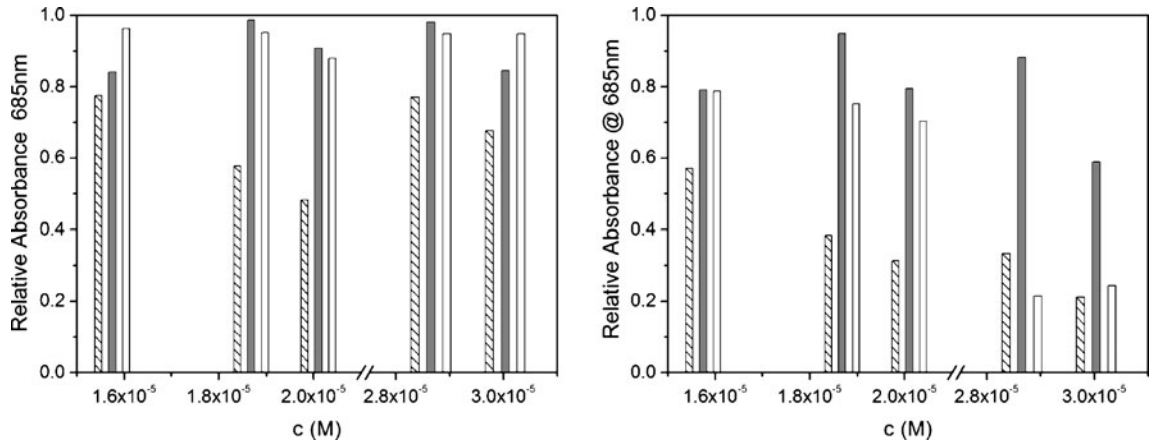

Fig. 3 Dye loss after polymerization at 323 K (*left*) and after subsequent annealing at 343 K (*right*), determined from the relative changes in absorbance at 685 nm for different concentrations of AIBN: 0.1w% AIBN (*patterned bar*), 0.5w% AIBN (*grey bar*), 1w% (*white bar*). The absorbance of the MMA solution prior polymerization was set to one

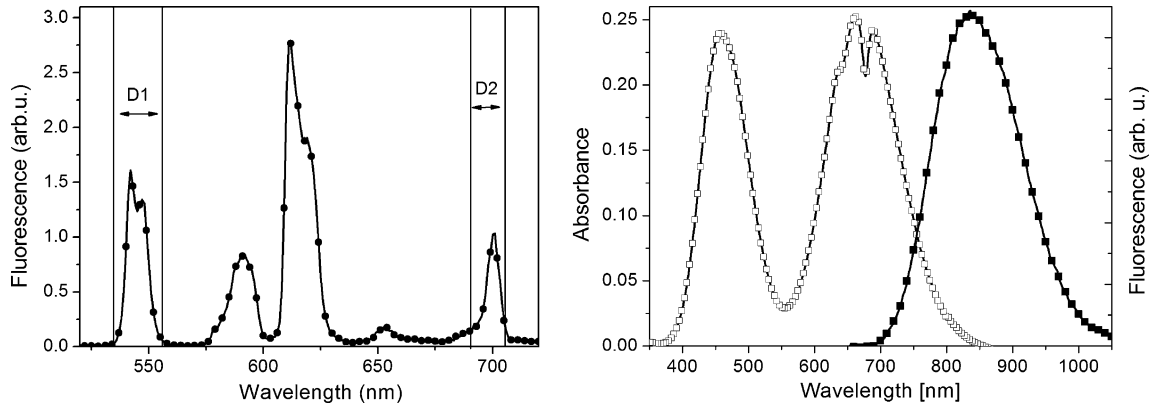

Fig. 4 Left: Emission spectrum of a rare earth-doped glass (ME-glass, *solid symbols*). Right: Absorption (*open symbols*) and emission spectrum (*solid symbols*; excitation at 590 nm) of a Cr^{3+}-doped glass. The glass thickness and thus the optical pathlength was 1 mm

Homogeneity

Analysis of the emission spectra of the single component-polymer systems recorded during the x,y-scan as well as mappings of slices prepared from polymers doped with a single dye revealed different dye distributions for F305 and Rh800, respectively (see Fig. 5a and b). The fluorescence intensity of Rh800 detected in the wavelength region D2 (see Fig. 2) decreases continously from the edges to the middle of the slices while the distribution of F305 is rather homogeneous. To determine the homogeneity of the relative dye distribution in the dual doped polymer systems, we calculated the quotient Q of the integral fluorescence intensities D1 and D2 (Q=D1/D2). The detection ranges D1 and D2 in the long wavelength regions of the emission bands of the dyes were chosen to minimize the influence of reabsorption effects on the examined signals. To illustrate the relative intensity deviations along the x- and y-direction of the studied sample (see Fig. 5c), the maximum value of Q was set to 1. As follows from Fig. 5c, the observed variations in fluorescence intensity are in the order of 2%. Mapping results of slices cut along the z-direction of a representative sample are presented in Fig. 5d–f. The dye distribution along the z-direction of the polymer differs

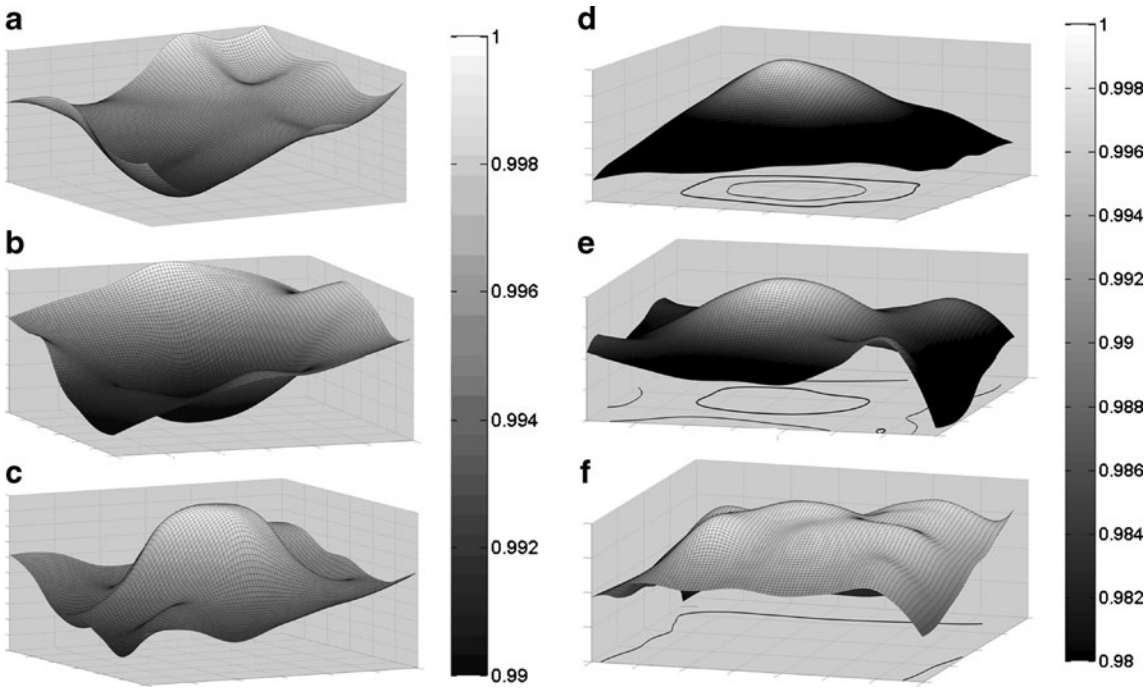

Fig. 5 **a** Emission intensity detected in D1 (see Fig. 2, *right*), **b** emission intensity detected in D2 (see Fig. 2, *right*) in the x- and y-plane of a dual dye-doped polymer slice (7 mm×7 mm×1 mm). **c** Quotient of D1 and D2 (Q=D1/D2). **d–f** Quotient Q measured along polymer slices (8 mm×8 mm×4 mm) originating from different z-positions within the dual doped polymer block. The contour plot in the x,y-plane indicate the 1% and 2% limits, respectively

only slightly. In the bottom layer (Fig. 5f), both dyes are uniformly distributed with deviations of Q in the order of 1%. A layer from the mid-area of the cuvette (Fig. 5e) shows deviations of about 2%. Mapping of the top slice of the cuvette-shaped polymer block (Fig. 5d) reveals a deviation of the calculated signal ratios <1% for about 10% of the mapped area and deviations <2% for an area amounting to about 25% of the overall mapped area. These results underline the sufficient homogeneity of the dye distribution in this two component material.

For comparison, the homogeneity of the emission of the ME glass (see Fig. 4) was examined, assessing the integral fluorescence intensities of the emission bands of Tb^{3+} and Er^{3+} (see spectral regions D1: 530 nm to 565 nm (Tb^{3+}; $^5D_4 -> ^7F_5$) and D2: 680 nm to 715 nm (Er^{3+}; $^5D_0 -> ^7F_4$) in Fig. 4). Evaluation of the quotient Q=D1/D2 yields a relative deviation of the ratio of the integral fluorescence intensities below 0.25%, thereby indicating the excellent homogeneity of the fluorophore distribution. Nearly the same homogeneities were observed for a Cr^{3+}-doped glass when evaluating the integral fluorescence intensity within the wavelength region between 750 nm and 1000 nm. Although the homogeneity of the chromophore distribution in the glasses is better than the homogeneity found for the two component polymer material, it needs to be kept in mind here that in the case of the glass standard candidates, these cuvette- or slide-shaped materials were always obtained from a bigger glass block, the edges of which had been removed in a first automatic processing step. A similar processing step will result in homogeneities in relative flourophore distribution in the order of ≤ 1% for our two component polymer materials.

Anisotropy

Figure 6 (left) compares the emission anisotropy of a PMMA block doped with F305 and Rh800 with the emission anisotropies obtained for a commercial PMMA block containing rhodamine B, an ethanolic solution of rhodamine B as well as the anisotropies of ME- and Cr^{3+}-doped glasses. The dual doped polymers display wavelength-dependent emission anisotropies. In the range of the F305 emission between 575 nm to 635 nm, the anisotropy shows a value r_{F305} of about 0.4, the maximum theoretical value and equals the emission anisotropy displayed by rhodamine B in PMMA determined between 550 nm and 650 nm (Fig. 6, left panel), whereas in the emission range of Rh800 (700 nm to 900 nm), a reduced anisotropy of about $r_{Rh800} \approx 0.32$ was observed (Fig. 6, right panel). Contrary to the dye-doped polymeric systems the emission anisotropies (r ≈ 0.01) of the Cr^{3+}-and ME-doped glasses are comparable to the emission anisotropy of rhodamine B in ethanolic solution (r ≈ 0.03).

For the suggested application of the dye-doped PMMA systems as fluorescence intensity standards, the high emission anisotropy does not present a problem, even for instruments dispensing with polarizers. However, the emission anisotropies > 0.05 generally found for organic dyes incorporated into polymeric systems limits the merit of such materials for the use as spectral fluorescence standards, since this can result in an enhanced uncertainty for the determination of the spectral responsivity, especially for instruments lacking polarizers, compared to standards revealing only small emission anisotropies [25, 26]. Here, fluorescent glasses like the Cr^{3+}-doped system or liquid standards like F001 to F005 are to be favored.

Stability studies

To assess the photostability of the dual doped polymer systems, 11 different slices made from these materials were illuminated under identical conditions (excitation wavelength 590 nm, irradiance of 0.15 mW/mm^2). Promisingly, under the conditions chosen for this proficiency test, the

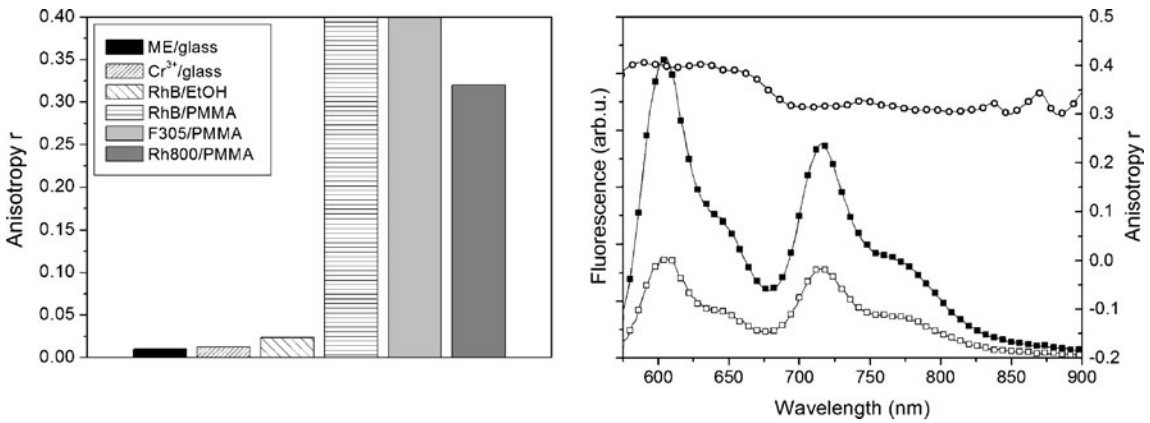

Fig. 6 Left: Emission anisotropies of a dual dye doped (F305 and Rh800) and a RhB doped polymer block in comparison to RhB in ethanolic solution and to ME-as well as Cr^{3+}-doped fluorescent glasses; Right: Emission anisotropy as a function of emission wavelength measured for a two-component polymer block

photostability of the dual doped polymer materials is comparable to that found for the Cr^{3+}-doped glass. No photodegradation was observed during 16 h of irradiation. To obtain a first hint for the thermal stability of the two component-systems, the samples were reheated after the preparation (see "Optimization of polymerization conditions") to 343 K for 23 h. This heating step leads only to a very small extra dye loss. We observed only a decrease in absorbance (measured at 685 nm), and thus in Rh800 concentration, of about 2.5%±1.4%. For a first insight into the longterm stability of the dye-doped polymers, three different single dye-doped cuvette-shaped polymer blocks were prepared and their application-relevant spectroscopic properties were measured over a period of 157 days. In between these measurements, the samples were stored at room temperature in the dark. Promisingly, we did not observe a change in the emission features of these samples within the range of the corresponding measurement uncertainty.

The results from the photochemical, thermal, and long term stability studies underline the potential of these polymeric systems as candidate intensity standards. At present, more rigorous longterm stability studies are being performed with these materials focusing on specific applications.

Conclusions

With the presented simple polymerization procedure, we identified a straightforward and inexpensive method for the fabrication of homogeneously dye-doped polymers suitable for single and two component systems. This enables the production of multi-emissive polymer-based fluorescence intensity standards for the vis and NIR spectral region with homogeneous application-relevant spectroscopic properties and an excellent photochemical and thermal stability. The dual-emissive intensity standards based on F305 and Rh800 enable ratioing of (integral) emission intensities for excitation wavelengths between 400 nm and 750 nm. The ratios of the detected fluorescence intensities within the emission region of ca. 550 nm to 850 nm are tunable via dye concentration and variation of the excitation wavelength. With the additional use of stable dyes with high quantum yields from e.g. the Lumogen family absorbing and emitting at shorter wavelengths the spectral region of emission can easily be extended to the deep blue part of the vis region [44]. The potential of these materials as external and built-in fluorescence intensity standards and instrument validation standards is currently being investigated exemplary for miniaturized multi-channel fluorescence sensors.

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