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Determination of Certified Values and Uncertainties for SRM 2942a

Relative Intensity Correction Standard for Fluorescence Spectroscopy: Ultraviolet Emission

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Abstract

Standard Reference Material (SRM) 2942a is a cuvette-shaped, Ce-ion-doped glass, recommended for use for relative spectral correction of emission from 320 to 430 nm and day-to-day performance verification of steady-state fluorescence spectrometers. It was certified for relative intensity of fluorescence as a function of emission wavelength. SRM 2942a is a renewal of SRM 2942, which was originally released by NIST in 2009. The techniques and methods used to determine the certified values and uncertainties are described here. Material properties that were explored include photostability, absorbance, dissolution rate in water, anisotropy and temperature coefficient of fluorescence intensity. The expanded uncertainties in the certified spectrum are about 5% around the peak maximum at 330 nm, using an excitation wavelength of 310 nm. The SRM also exhibits a strong resistance to photodegradation.

Keywords

calibration, cerium glass; fluorescence; instrument qualification; spectral correction; spectrometer; SRM; standards.

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1. Introduction

SRM 2942a has been certified for relative fluorescence intensity as a function of emission wavelength, i.e., spectral shape of emission. The general procedure used to collect fluorescence spectra, as part of the certification, is given here, along with how all data were combined to get the certified values and how corresponding uncertainties were determined. The certified values of SRM 2942a are intended to be used to obtain correction factors as a function of emission wavelength for the detection system responsivity of fluorescence spectrometers from 320 nm to 430 nm. SRM 2942a also exhibits excellent photostability under lamp irradiation at wavelengths longer than 280 nm. Similar procedures for certification and photostability determination of SRM 2942 were reported previously. [1]

2. Experiment

A more detailed experimental description of many of these procedures has already been reported. [2]

2.1 Samples

Ce-doped (0.00025 % by mole) phosphate glasses, made by Starna Scientific Ltd. using the procedures given in the *Glass Production Method* section of this report were cut and polished into cuvette-shaped pieces (12.5 mm x 12.5 mm x 45.0 mm). Four different glass blocks from four different melts, corresponding to four different batches were used to produce SRM 2942a. Slight differences in the emission spectra between batches 1, 2 and 3 have resulted in batch certified values for Lots 1, 2 and 3, respectively. The emission spectra for batches 3 and 4 are almost identical, resulting in our pooling of the data for batches 3 and 4 giving certified values for Lot 3 (comprised of both batches 3 and 4). The different Lots will be identified in the SRM's name, i.e., SRM 2942a Lot 1, Lot 2, Lot 3.

2.2 Fluorescence Spectrometry Method

A SPEX Fluorolog 3 (Jobin Yvon) fluorescence spectrometer was used to measure the fluorescence spectra of a sampling of units for each batch. The qualification and validation of this high accuracy fluorescence spectrometer has been described previously [3]. Each sample was measured four times, twice in normal position and twice when raised 7 mm to test spatial homogeneity. The samples were measured at 25 °C and regulated using a temperature controlled single-cuvette holder. The holder has a thermocouple within it that is used to feedback-adjust the water temperature of the circulation bath, to maintain the desired temperature. For measuring SRM 2942a, the following parameters were used, excitation (EX) wavelength (λ_{EX}) = 310.1 nm, emission (EM) wavelength (λ_{EM}) = 320 nm to 430 nm, increment = 1 nm, bandwidths of 3 nm for excitation and emission, integration time of 0.5 seconds for spectra and 5 seconds for single point collection, photomultiplier tube (PMT) voltage = 950 V. A small fraction of the excitation beam is diverted to a “reference” photodiode just before the sample to monitor the relative excitation intensity simultaneously with the signal intensity measured at the PMT. The ratio of signal and reference intensities is given as the “fluorescence intensity” in what follows to correct for signal intensity fluctuations due to changes in the excitation intensity. A calibrated light source (CS) was used, along with a calibrated reflector placed at the sample position to steer the light from the CS into the detection system, to measure the correction for the responsivity of the detection system to be applied to the fluorescence spectra measured as part of the certification. This correction was measured twice a day, just before and just after SRM samples were measured. The emission monochromator was scanned from 320 nm to 430 nm with 1 nm increment, other instrument settings were the same as used for collecting the certification data.

To measure the uncertainty in the certified values due to excitation wavelength uncertainty (± 0.2 nm) fluorescence spectra were measured at excitation wavelengths from 306 nm to 314 nm for SRM 2942a. To measure the uncertainty in the certified values due to excitation bandwidth uncertainty (± 0.2 nm), fluorescence spectra were measured at excitation bandwidths of 2 nm, 3 nm and 4 nm with the emission bandwidth fixed at 3 nm.

2.3 Glass Production Method

All glasses were melted under forming gas (95 % N_2, 5 % H_2) using alumina (Al_2O_3) vessels and electrically-heated air-atmosphere furnaces. The melts were stirred for homogeneity using a motor-driven fused silica propeller-type stirrer. A glass (base: 50 mol % P_2O_5, 47 mol % CaO, and 3 mol % Al_2O_3) with a suitable amount of dopant glass (0.25 mol % CeO_2, see Table 1) was prepared. The latter was approximately the same composition as the base glass (see Table 2), but contained the active ingredient (CeO_2) at about 100 times the desired final concentration.

Table 1. Preparation of Ce-Dopant (“Essence”) Glass.

Ingredient	Wt %	Mol %	Amount used, g
P_2O_5	70.48	50.00	23.5688 g $Ca(H_2PO_4)_2H_2O$ + 1.4960 g NH_4 H_2PO_4
CaO	26.04	46.75	1.4960 g NH_4 H_2PO_4
Al_2O_3	3.04	3.00	0.6122 g Al_2O_3
CeO_2	0.439	0.257	0.0884 g CeO_2

The ingredients were weighed into a suitable jar, mixed thoroughly on a rotary tumble mixer, transferred to an alumina crucible, heated to 1200 °C, stirred, and poured into water. After cooling, the glass cullet was more finely crushed in an alumina mortar and pestle.

Table 2. Preparation of Ce-Glass.

Ingredient	Wt %	Mol %	Amount used, g
P_2O_5	70.68	49.98	1184.6 g $Ca(H_2PO_4)_2H_2O$ + 67.87 g NH_4 H_2PO_4
CaO	26.27	47.02	67.87 g NH_4 H_2PO_4
Al_2O_3	3.05	3.00	30.56 g Al_2O_3
CeO_2	0.00044	0.00026	0.9990 g CeO_2 Essence glass

The ingredients were weighed into a suitable jar, mixed thoroughly on a rotary tumble mixer, transferred to an alumina crucible, melted at 1300 °C, stirred for one hour, and held at 1300 °C for 18 hr without stirring. The melt was cooled to 1200 °C and allowed to sit for 30 minutes before pouring. The melt was cast into an aluminum block-form mold 10 cm X 15 cm X 1.9 cm, preheated to 505 °C, and then transferred quickly to an annealing oven where the block was equilibrated and soaked at 505 °C for 8 hours. The annealing oven was then ramped down from 505 °C to 240 °C at 10 °C/hr and from 240 °C to room temperature at 30 °C/hr (required ≈ 40 hr).

2.4 Cutting and Polishing of Glass Artifacts

The four batches of glass were cut and polished into rectangular, standard-cuvette-shaped artifacts. Fabrication specifications:

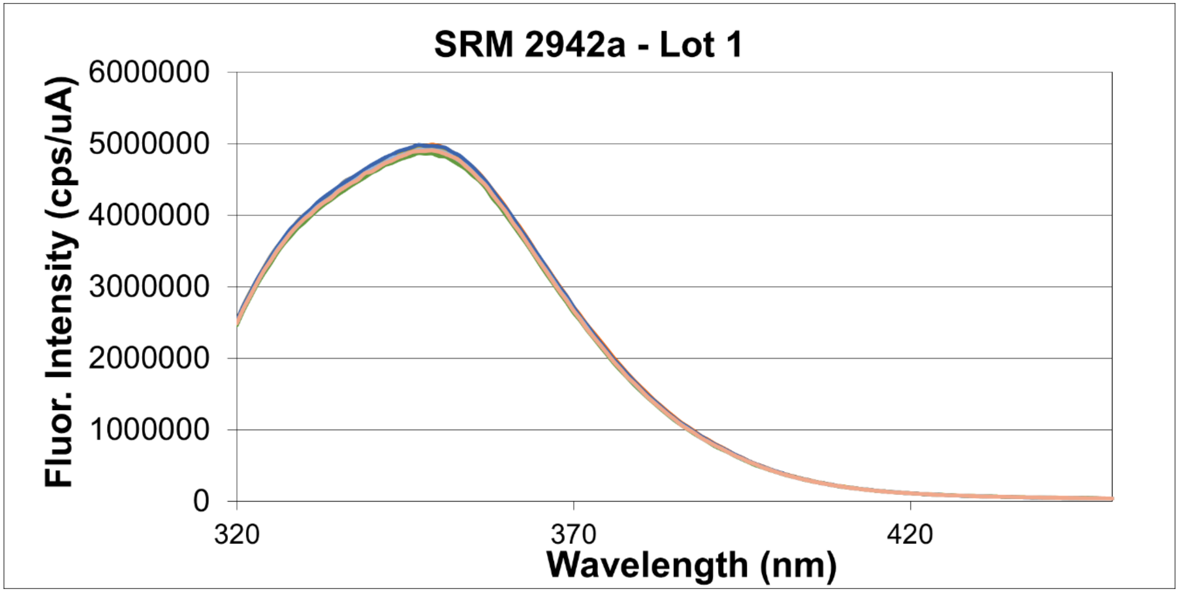
- dimensions 12.5 mm +/- 0.1 mm by 12.5 mm +/- 0.1 mm by 45 mm +/- 1 mm
- three 12.5 mm by 45 mm surfaces to be polished
- finish scratch and dig tolerance 60/40
- polished surfaces flat to 4 fringes at 633 nm
- polished surfaces parallel to 3 arc minutes
- both 12.5 mm by 12.5 mm surfaces (top and bottom) and one 12.5 mm by 45 mm surface to be frosted (400 grit alumina sanding) to give 1 micron to 3 micron rms surface roughness
- slight 45 degree chamfer to be applied to each 12.5 mm edge

3. Results and Discussion

3.1 Certification Data

A difference of less than 1% was observed between normal and raised position samples, implying that all SRM samples are spatially homogeneous on a millimeter to centimeter scale. The fluorescence intensity of all spectra within a particular lot had a standard deviation across the entire wavelength range of about ≤ 2% for Lot 1, ≤ 4% for Lot 2, and ≤ 3% for Lot 3.

A



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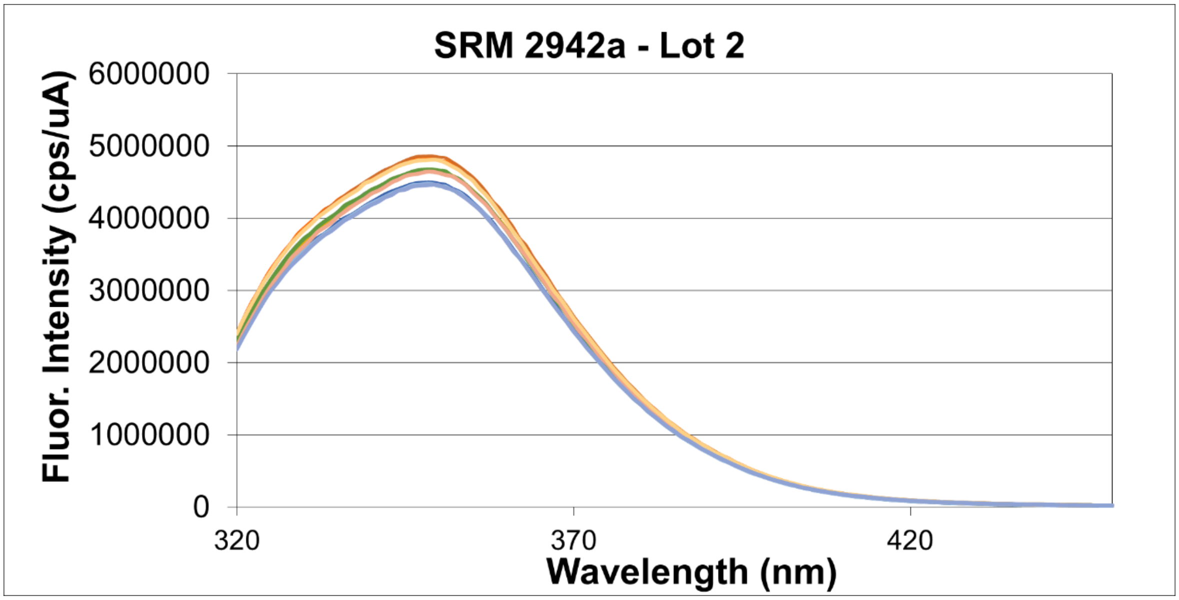

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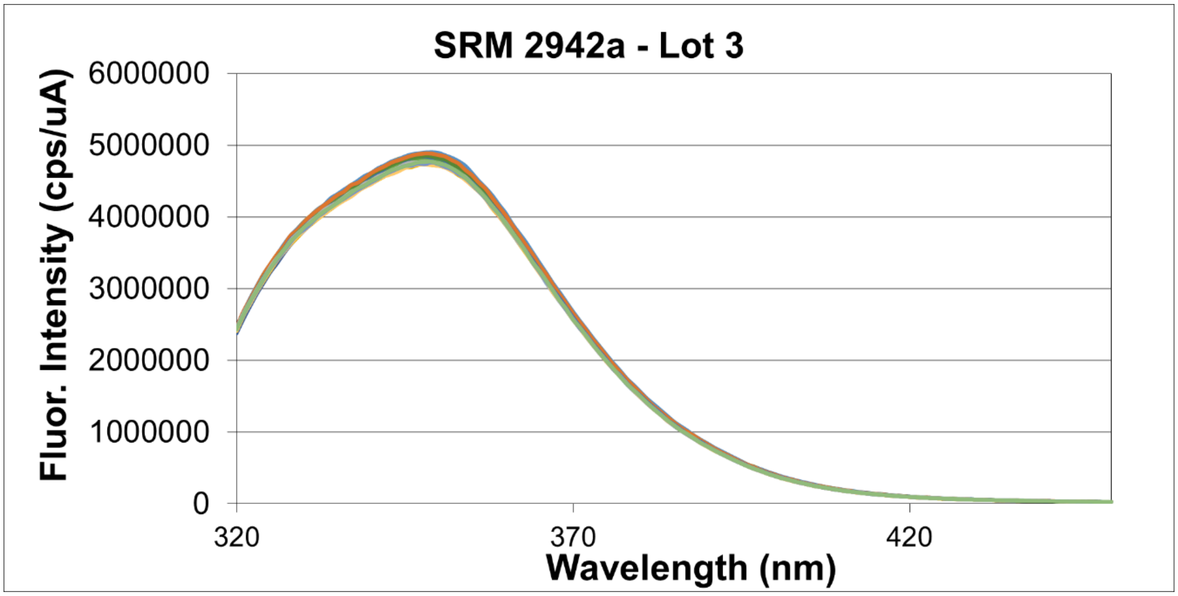


Fig. 1. Raw EM spectra (not spectrally corrected) for measured units of SRM 2942a by lot.

The peak maximum intensity for the uncorrected fluorescence spectra was observed at about 350 nm for all three lots with the intensity for Lot 2 being 5% less and Lot 3 being 2% less than Lot 1 (see Fig. 1A-1C). The final fluorescence spectrum for each sample was obtained by

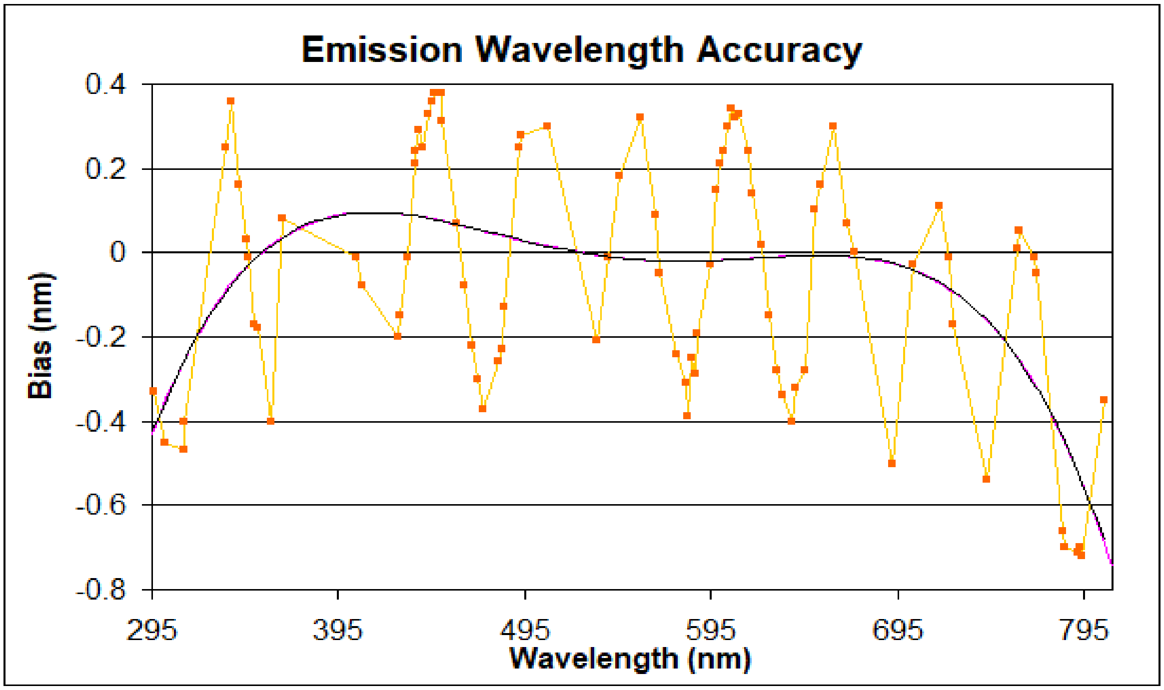
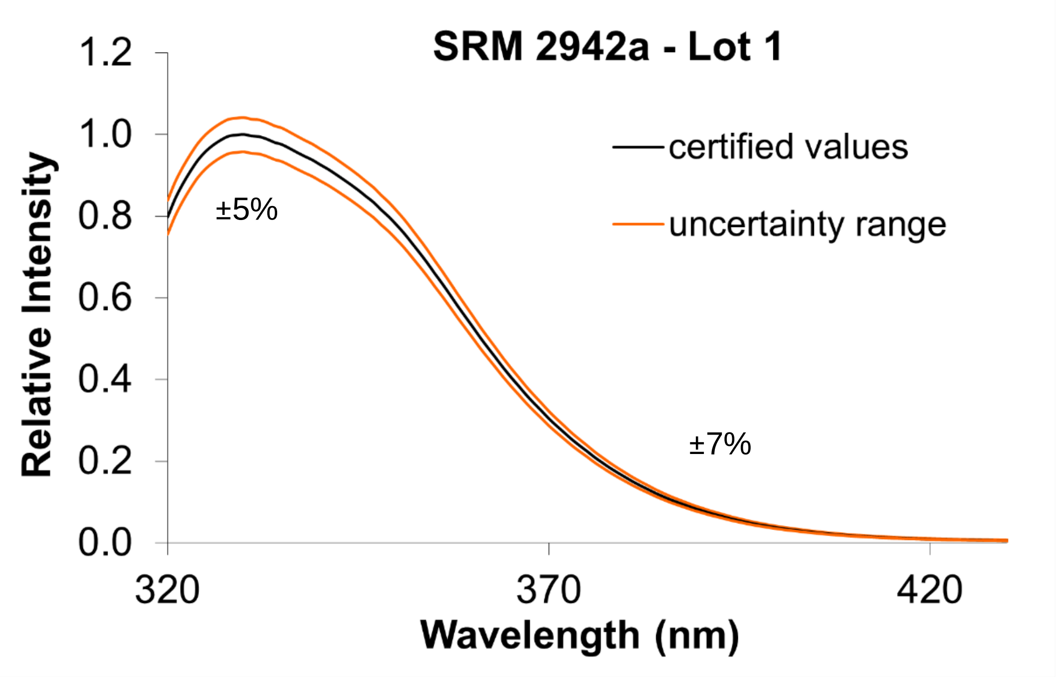


Fig. 2. Emission wavelength accuracy bias of the fluorescence spectrometer.

averaging the beginning and end of day correction factors, giving one spectrum and one correction factor for each sample. The spectrum was then multiplied by the correction factor to give a shape-corrected spectrum. The shape-corrected spectra from all samples in a particular lot were then averaged to give a single shape-corrected spectrum for each lot. A small emission wavelength bias (≤ 0.4 nm) that changes as a function of wavelength was observed on the Fluorolog 3 (see Fig. 2). Correcting for this bias was not necessary in the region from 320 nm to 640 nm, but a 1 σ uncertainty of ± 0.2 nm was included in the total uncertainty calculation. Since these SRMs were certified for relative, not absolute, intensity, the certified values were normalized to one at 330 nm for all lots of SRM 2942a, for ease of use. The certified relative fluorescence intensities and total uncertainties for all lots are nearly indistinguishable (see Figs 3A-3C). Each lot will have its own certificate with certified values and uncertainties due to the differences in absolute fluorescence intensities and standard deviation for each lot.

A



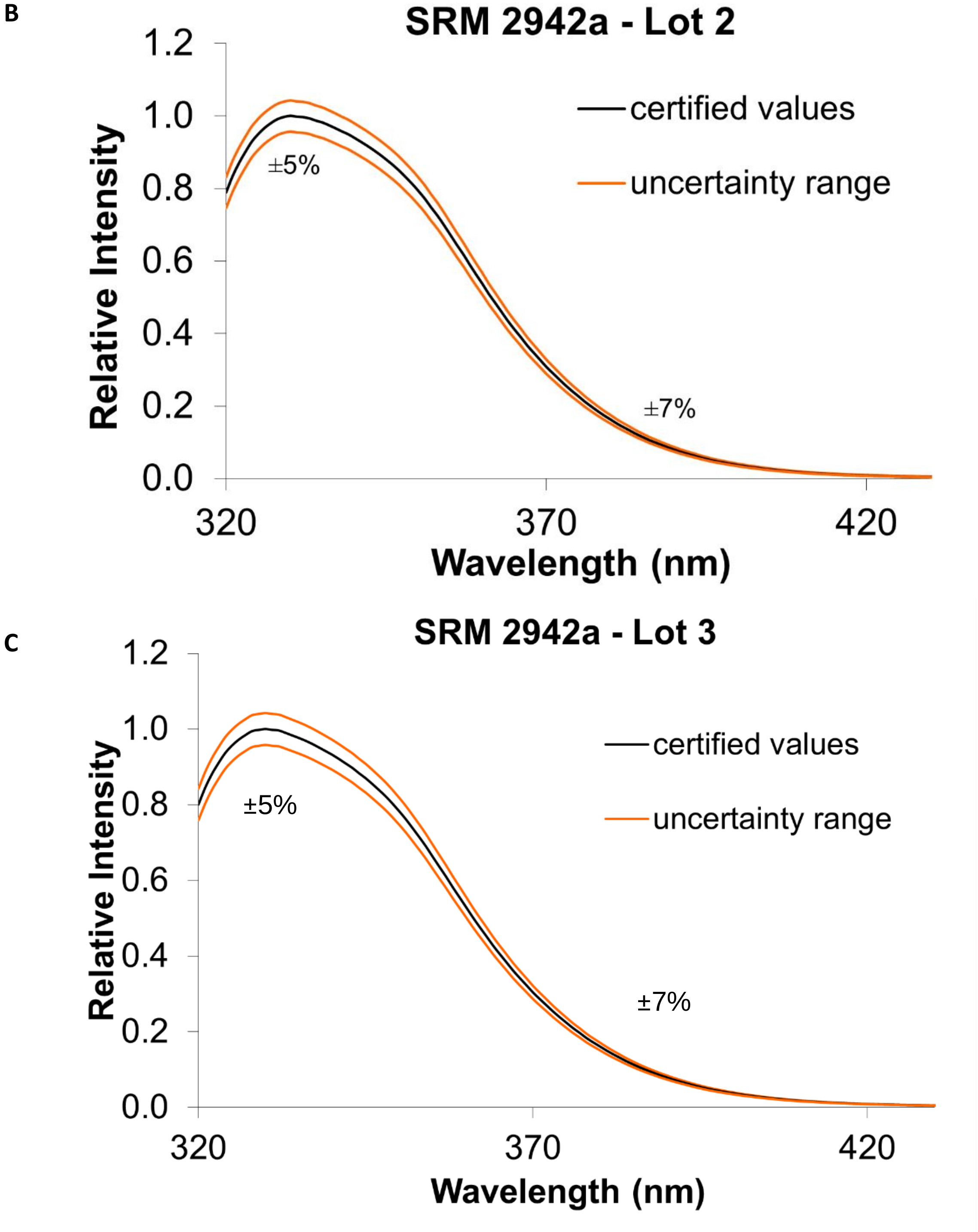


Fig. 3. Certified EM spectra and expanded uncertainties for SRM 2942a by lot.

3.2 Excitation Wavelength Uncertainty

The excitation spectra for SRMs 2942a (peak max at 296 nm) have FWHM of about 28 nm (see Fig. 4). The emission spectrum exhibited a relatively linear peak shift and % diff when shifting excitation wavelength away from the excitation peak max (see Fig. 5A). Differences of 1 % to 10% were observed with an excitation wavelength shift of 1 nm. The % diff corresponding to a 1 nm shift for SRM 2942a (see Figure 5B) was fitted to a polynomial which was used to calculate the uncertainty in the certified values due to the excitation wavelength uncertainty (± 0.2 nm) by dividing the polynomial values by the appropriate factor, 5 for SRM 2942a.

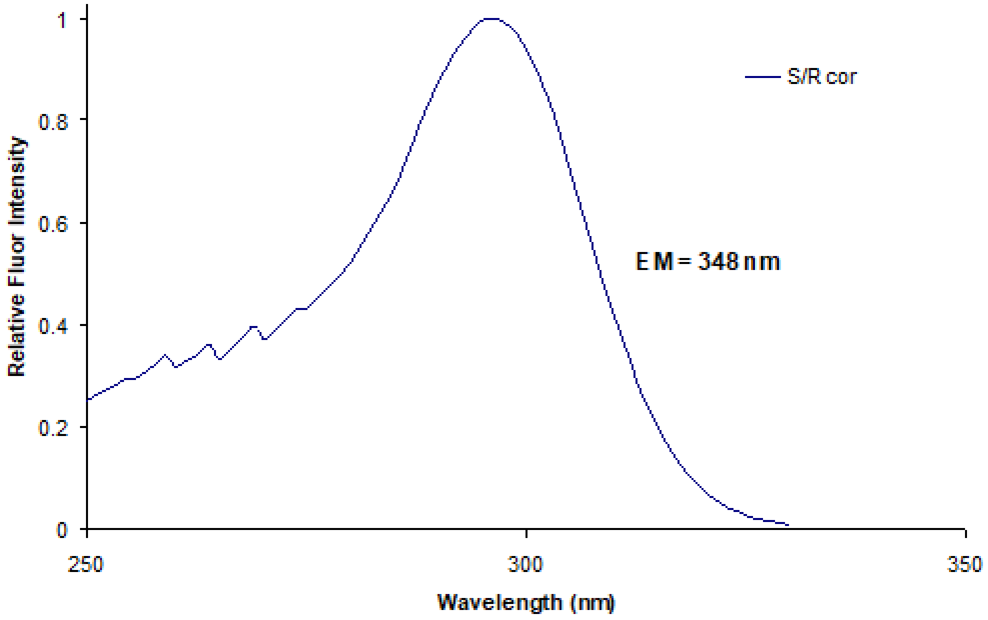


Fig. 4. Fluorescence EX spectrum of SRM 2942a.

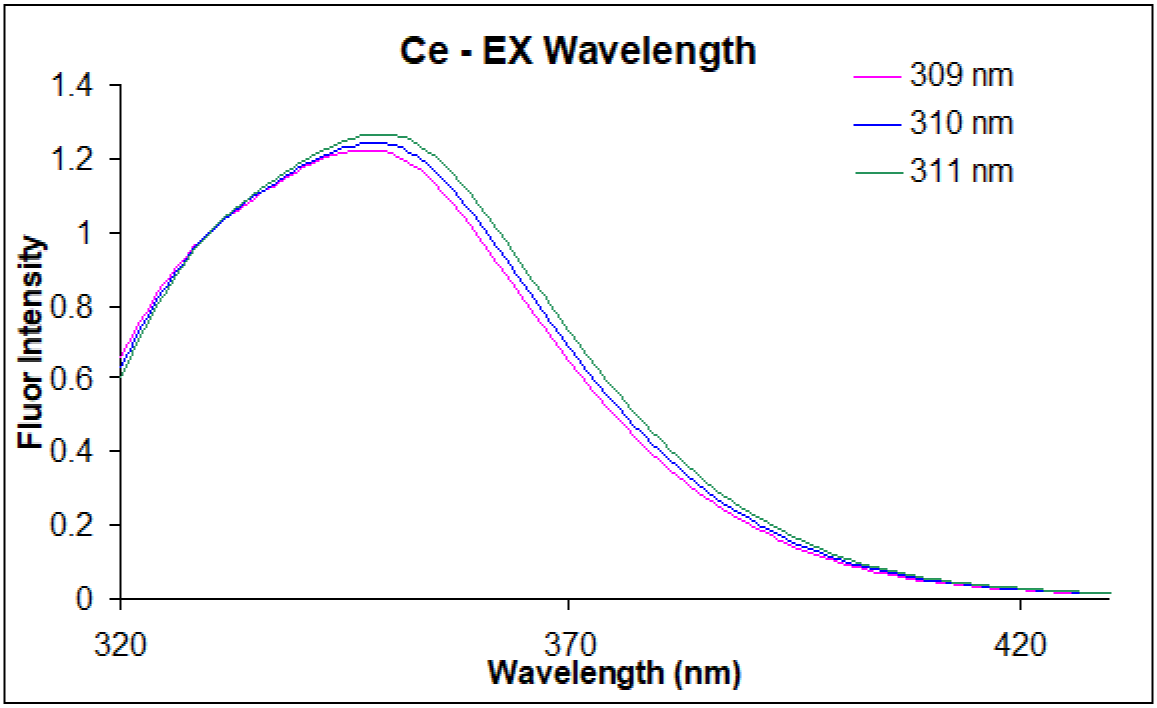


Fig. 5A. Fluorescence EM spectra of SRM 2942a at different excitation wavelengths.

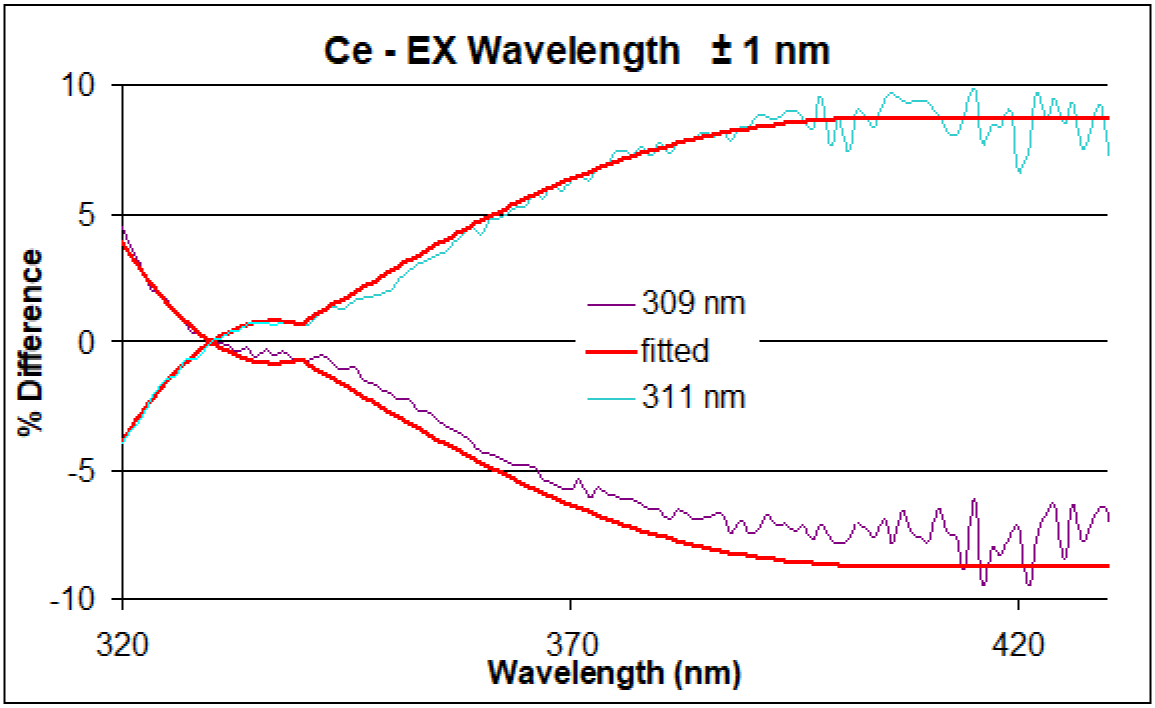


Fig. 5B. Percent difference in fluorescence excitation spectrum of at different EX wavelengths.

3.3 Excitation Bandwidth Uncertainty

The percent difference of the relative fluorescence intensity as a function of excitation bandwidth was calculated from the collected spectra. A maximum percent difference of less than 3 % was found for SRM 2942a when the excitation bandwidth was changed by 1 nm in either direction. The spectrum corresponding to the direction with the slightly larger % diff was used to calculate the uncertainty in the certified values due to the excitation bandwidth uncertainty (± 0.2 nm) by fitting a treadline to the % diff and dividing by 5 (see Fig. 6).

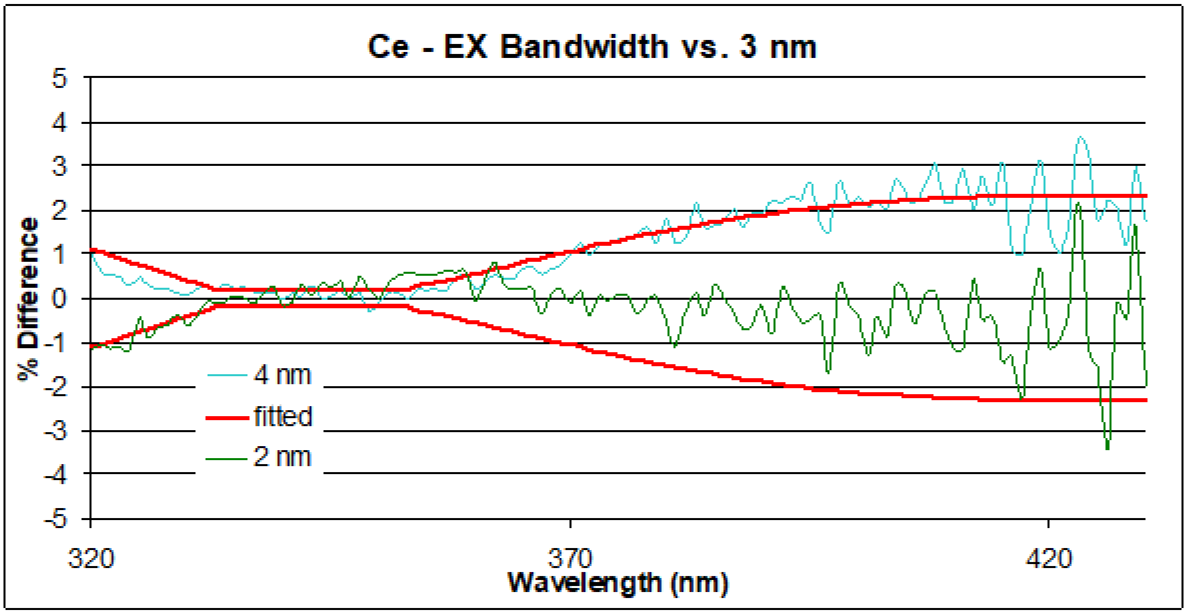


Fig. 6. Percent difference in fluorescence EM spectrum at different excitation bandwidths.

3.4 Emission Bandwidth Uncertainty

The percent difference of the relative fluorescence intensity as a function of emission bandwidth was calculated using a method developed by Mielenz [4] using numerical differentiation of the collected emission spectrum at 3 nm bandwidth in this case. A maximum percent difference of about 0.3 % was found when the emission bandwidth was changed by 1 nm in either direction. The % diff spectrum was used to calculate the uncertainty in the certified values due to the emission bandwidth uncertainty (± 0.3 nm) by fitting a trendline to the % diff and multiplying by 0.3 (see Fig. 7).

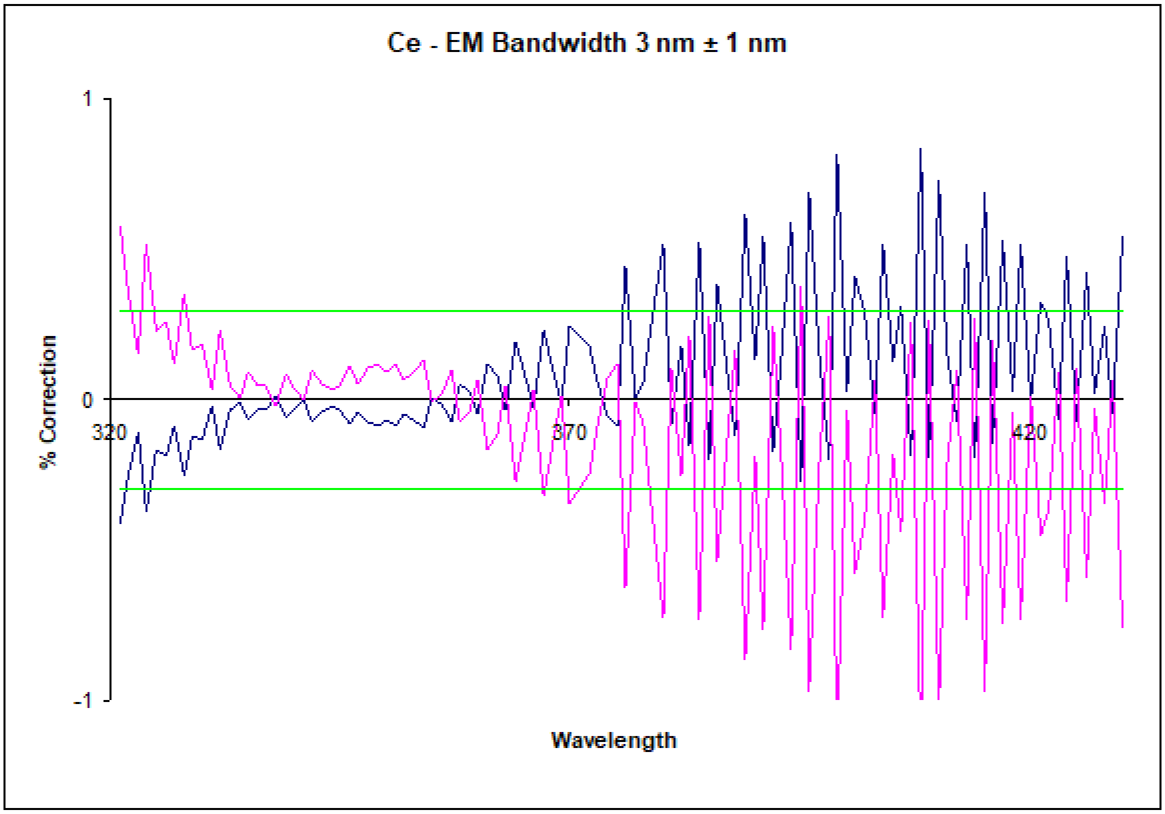


Fig. 7. Percent difference in fluorescence EM spectrum at different EM bandwidths.

3.5 Emission Wavelength Uncertainty

The percent difference of the relative fluorescence intensity as a function of emission wavelength shift was calculated by taking the certified intensity values and shifting them by 1 nm in both directions. The % diff was found to be ≤ 5 % throughout most of the spectrum (see Fig. 8). The spectrum corresponding to the direction with the slightly larger % diff was used to calculate the uncertainty in the certified values due to the excitation wavelength uncertainty (± 0.2 nm) by fitting a trendline (polynomial) to the % diff and dividing by 5.

3.6 Inner Filter Effect Uncertainty

The alignment error due to a sideways shift of the viewed sample volume (likely due to a sideways shift of the excitation beam) causing a change in the self-absorption of the fluorescence emission of the sample (secondary inner filter effect) is given by Eq. (1)

$$S = 10^{(\varDelta / W)[A(\lambda_m) - A(\lambda_{m,0})]}, \quad \varDelta = b - W / 2 \qquad (1)$$

where $\varDelta$ is the misalignment of the viewed sample volume in the direction of viewing, defined as the deviation of the distance b (from the incident surface of the sample to the

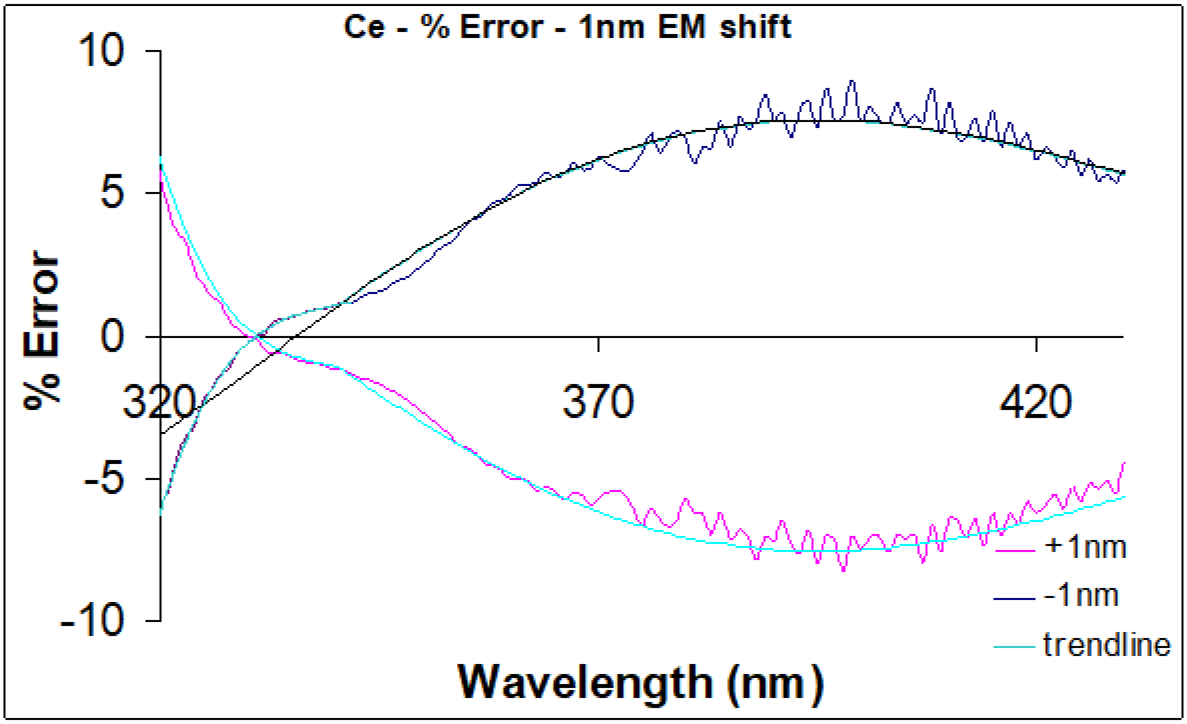


Fig. 8. Percent difference in fluorescence EM spectrum with a shift in EM wavelength.

detection region's center) from its standard value *W*/2, where W is the width of the sample. It is again noted that the corresponding error due to a misalignment of the detection region in the

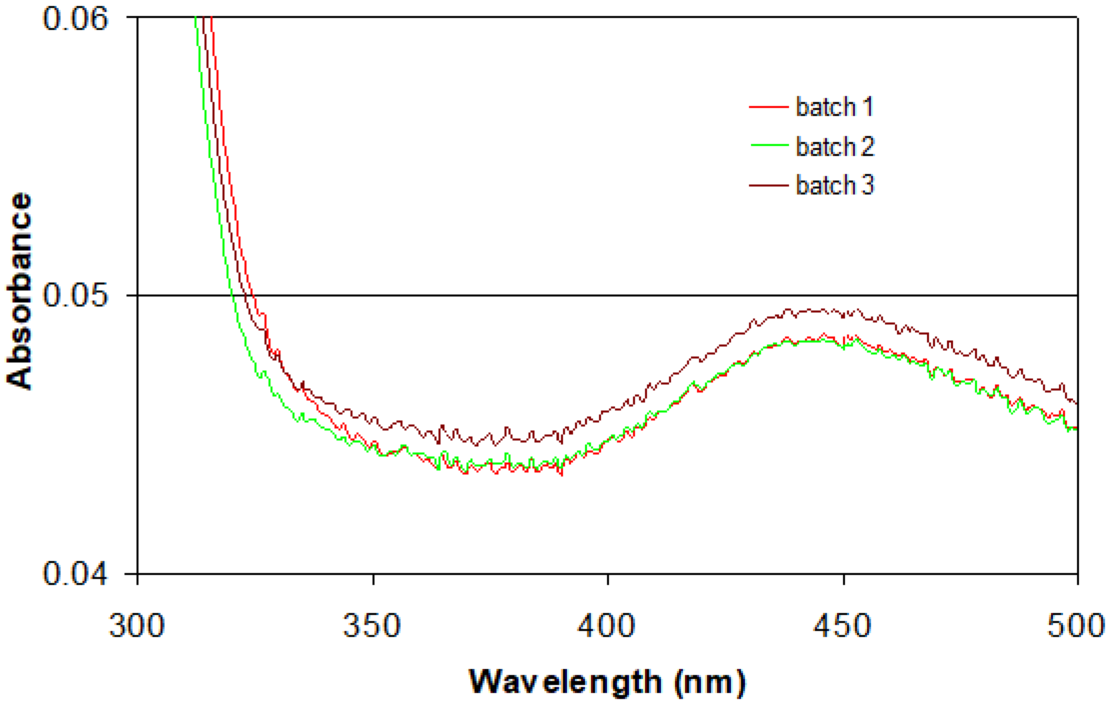


Fig. 9: Absorbance spectra for different batches of SRM 2942a.

direction of the excitation beam causing a change in the absorption of the excitation beam (primary inner filter effect) is eliminated by the normalization of the emission spectrum.

The absorbance spectra of all batches of SRMs 2942a are similar and all values are relatively small, $A \leq 0.04$ (see Fig. 9). The certified relative intensity values for SRM 2942a were measured at the center of the sample with a position uncertainty of ± 1mm. This results in insignificant inner filter errors (see Fig. 10) in the certified values (≤ 0.1 %).The sample shift error values for the different batches will be included in the certified uncertainty values.

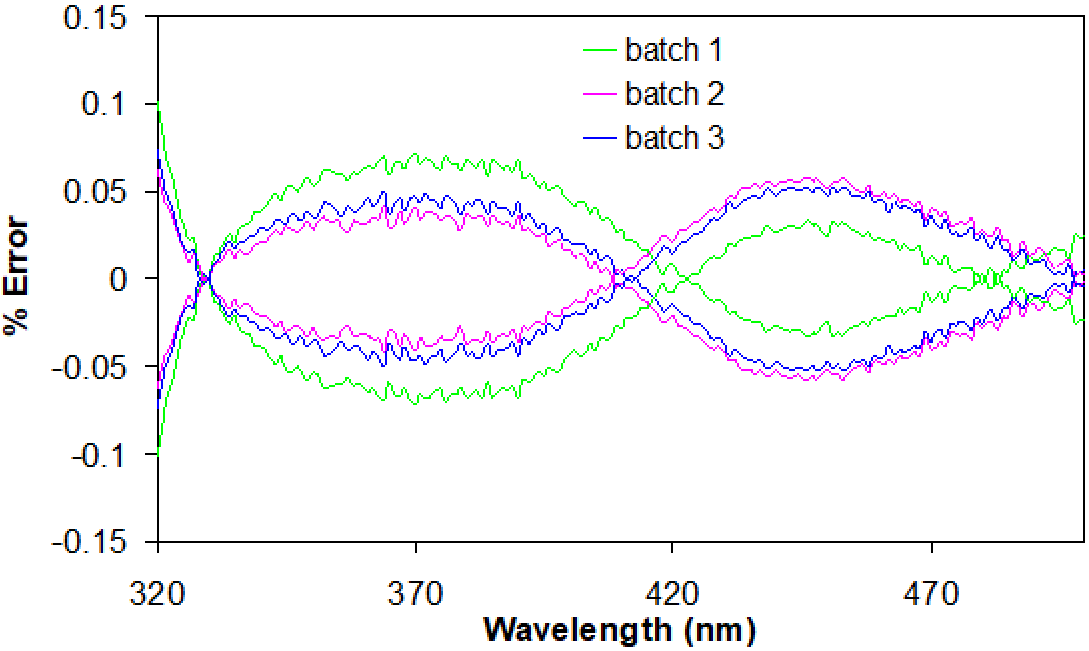


Fig. 10. Sample shift errors due to inner filter effects.

3.7 Polarization Uncertainty

The polarization ratios of the excitation beam and emission detection system, or F and G factors, respectively, are defined in Eq. (2)

$$F = \frac{\Phi^V}{\Phi^H} \quad \text{and} \quad G = \frac{R^V}{R^H} \qquad (2)$$

where Φ^V and Φ^H are the vertically and horizontally polarized components of the excitation flux, R^H and R^V are the corresponding spectral responsivities of the emission detection system.

A contribution to the uncertainty in the certified relative intensity values for SRMs 2942a due to a typically expected difference between the F and G values of the user's instrument and those of the NIST instrument will be included in the certified uncertainty values. The typically expected difference has been set at ± 25% from those of the NIST instrument, which is estimated to be a typical difference for conventional fluorometers designed to cover the emission region from about 350 nm to 750 nm with greatest sensitivity. When the F and G values of the user's instrument differ by ± 25% from those of the NIST instrument, the calculated user bias will be less than 0.2 % for SRM 2942a (see Fig. 11). These calculated % error values will be included in the certified uncertainty without any smoothing or fitting.

3.8 Temperature Uncertainty

The values of the temperature coefficient is –0.022 % ± 0.017 % / °C for SRMs 2942a, at 25 °C. It is assumed that temperature is normally distributed (Gaussian) around any particular temperature setting with a 1σ limit of ± 0.5 °C.

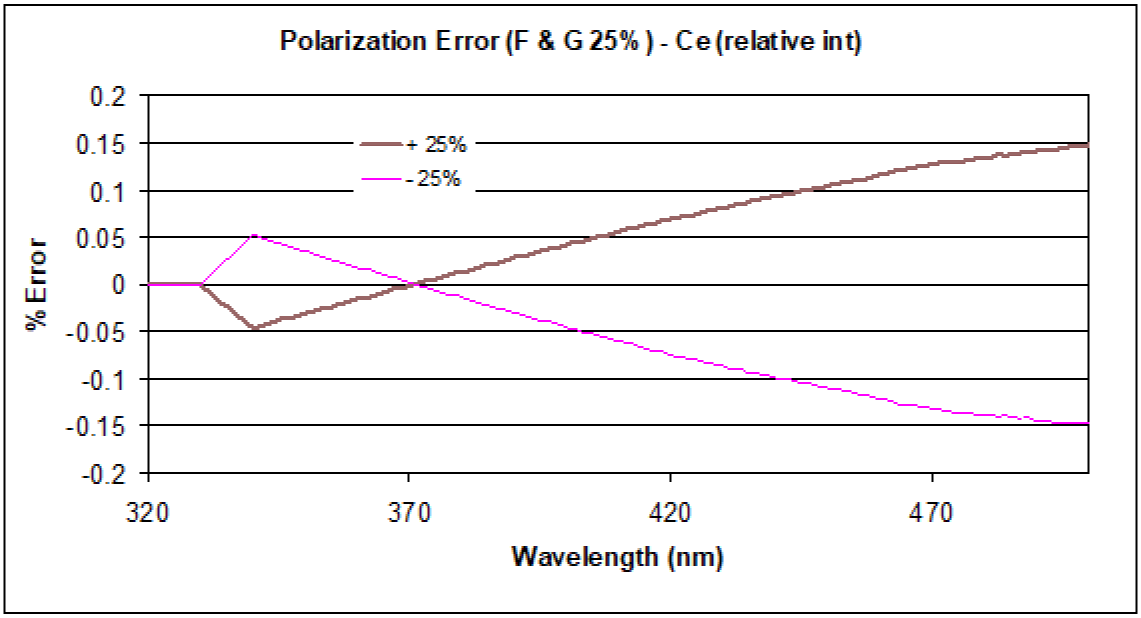


Fig. 11. Polarization Error.

Potential Error due to differences between instruments in polarization of the excitation beam and polarization dependence of the spectral responsivity of the detection system.

3.9 Photostability

After irradiating the SRM with a UV light source with a nominal intensity of 0.3 mW cm^{-2} nm^{-1} from 300 nm to 380 nm for more than 25 hours, no change in the absolute intensity or shape of the emission spectrum was observed within an uncertainty of ± 0.5 % (k=2) at the peak maximum. This amount of irradiation corresponds to about 50 hours of irradiation with our fluorometer's excitation beam under the conditions used for certification.

3.10 Instrument Stability over Time

Stability studies of the original SRM 2942 performed from 2008 to the present demonstrated that the SRM units are stable over time, within 3% across the certified spectra range, which is well within the certified uncertainties.

3.11 Certified Expanded Uncertainty

The total uncertainty in the certified relative fluorescence intensity values was calculated by adding in quadrature the 1σ uncertainties due to spatial uncertainty of the excitation beam's position on the sample (secondary inner filter effect uncertainties), instrument instability over time, variation of F and G polarization ratios between instruments (see ROA 831.02-06-103), temperature uncertainty (see ROA 831.02-06-105), excitation and emission wavelength and bandwidth uncertainty, uncertainty in the spectral shape correction (due to uncertainty in the radiance and reflectance values of the calibrated light source and reflector) and standard deviation of the certification data. The total uncertainty was calculated for each of the lots for the SRM. Note that only the last (standard deviation) of these uncertainties was found to be lot dependent. The total 1σ uncertainties were then multiplied by an expansion factor (k=2) to get the total expanded uncertainties (graphed in Fig. 3) at a 95% confidence level.

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