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Global analysis of time-resolved emission – a powerful tool for the analytical discrimination of chemically similar Zn^{II} and Cd^{II} complexes

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Abstract

The simultaneous analytical discrimination of spectrally very similar components with time- and wavelength-resolved fluorescence spectroscopy is demonstrated for the fluorescent probe $BP(OH)_2$ and its complexes with the d^{10} metal ions Cd^{II} and Zn^{II} in water. Whereas the absorption and emission spectra of the three components largely overlap the fluorescence lifetimes differ significantly. As a consequence, analyzing steady-state emission spectra of samples containing unknown amounts of both metal ions yields poor results for the analytical validity but the recording of fluorescence decay curves at different emission wavelengths improves the quality of the results drastically. The two techniques are compared in terms of goodness and analytical accuracy of the fit as well as analytical applicability.

Keywords: Time-resolved; Fluorescence; Zn^{II}; Cd^{II}; 2,2′-Bipyridyl-3,3′-diol

1. Introduction

The detection and quantification of heavy metal ions is of great importance in any kind of biological and environmental analysis. These ions affect biological systems in many different ways ranging from being essential trace elements to being acute toxins [1]. Whereas some heavy metal ions such as Cd^{II} and Hg^{II} are toxic for most biological organisms even at extremely low concentrations, ions such as Zn^{II} or Cu^{II} are often essentially involved in many biochemical reactions on a trace level, i.e., catalysis, transport, and biosynthesis [2]. However, at higher concentrations these ions cross the line between being essential to being toxic for an organism [1]. Due to many industrial production processes the anthropologically-disposed heavy metals are a major source of contamination in the environment [3]. In terms of environmental mobility and bioavailability, metal ions that are dissolved in water are amongst the most dangerous contaminants. They are easily transported in aquifers, taken up by plants or aquatic organisms, and they often interfere with any kind of dissolved organic or inorganic matter in water, e.g., humic substances [4–7]. Due to their similar electronic nature, some metal ions compete in biological uptake and transport processes and often contribute to either biological health or disease. One such prominent pair is examplified by Zn^{II} and Cd^{II}. For example, in many biochemical pathways Zn^{II} competes successfully with Cd^{II} and inhibits harmful reactions of Cd^{II}, e.g., the induction of tumors [1]. Therefore, the simultaneous determination of these chemically very similar ions is of great importance in analytical chemistry. Many techniques are known for the determination of these ions after sampling, preparation, and separation, however, the number of analytical methods making it possible to determine these ions simultaneously and in situ is rather small. Here, fluorescence spectroscopy employing fiber optics and sensors equipped with metal ion-sensitive fluorescent probes is a promising tool [8,9]. Furthermore, the limiting factors often encountered in the simultaneous determination of these ions in liquid solutions with steady-state fluorometry could be improved by employing time-resolved fluorometry.

The time-resolved recording of fluorescence spectra enables not only the simultaneous determination of spectrally very similar analytes having different fluorescence decay times but additionally allows for the temporal discrimination of scattered light and background fluorescence. Moreover, the detection of a certain fluorescence lifetime at a given wavelength contains two independent analyte-specific informations [8,10,11]. Within the last years, the

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number of applications of time-resolved fluorometry in environmental analysis of metal ions increased but to the best of our knowledge, no attempts have been made in utilizing the two-dimensional recording of the spectral and temporal emission matrix [12–24].

The fluorescent probe employed for the determination of Zn^{II} and Cd^{II} is the photostable and water soluble 2,2′-bipyridine [bipy] derivative 2,2′-bipyridyl-3,3′-diol [$BP(OH)_2$]. The spectroscopic behaviour of $BP(OH)_2$ was intensively studied both experimentally [22–35] and theoretically [36–40]. Whereas the high insensitivity to the influence of its environment in many organic solvents is well known, the spectroscopical data on $BP(OH)_2$ in aqueous solutions are sparse [22–26],[35]. In organic solvents, the emissive state was characterized as an ESIDPT (excited state intramolecular double proton transfer) state which fluoresces with a quantum yield of ca. 0.3 and shows a strongly Stokes-shifted emission spectrum [26–31]. In aqueous media, the spectroscopical behaviour differs significantly from that in organic solvents but the emission in the visible range of the spectrum is maintained over a large pH range (from pH 1 to 9) as well as in the presence of most common buffers. The interference of heavy metal impurities with the synthesis of $BP(OH)_2$ originally mentioned by Siemanowski et al. [41] and Naumann et al. [42] led to a more detailed investigation of the complexation-induced spectroscopical effects [22–25]. Only recently, Cargill Thompson et al. were able to isolate two different complexes of $BP(OH)_2$ with $Ru^{II}(bipy)_2$ [43] but these systems were only monitored in acetonitrile. Like its parent compound bipy, $BP(OH)_2$ forms stable complexes with various heavy and transition metal ions and thus is suitable as a fluorescent probe for these metal ions. The complexes formed with paramagnetic ions are non-fluorescent, whereas in the case of the diamagnetic heavy metal ions of the d^{10} group, only Hg^{II} forms a non-fluorescent but Zn^{II} and Cd^{II} form highly fluorescent complexes. The complexes of the latter two ions are only formed at ambient pH values [22–25]. Here, a comparative study of the fluorometric determination of the Zn^{II} and Cd^{II} complexes of $BP(OH)_2$ with steady-state and time-resolved fluorometry is presented, focusing on the global analysis of spectral and temporal emission data.

2. Experimental details

The fluorescent probe $BP(OH)_2$ was purchased from Aldrich, recrystallized from light petroleum, and checked for purity with HPLC. The analytical complexation studies were carried out with Zn^{II} and Cd^{II} sulphate from Aldrich and the corresponding perchlorates from ALFA of the highest purity commercially available. The salts were dried to a definite water content (dihydrates of the Zn^{II}/Cd^{II} salts: 100°C/120°C i. vac. for 12 h). Bidistilled water (pH 6.39) was provided by the Laboratory for Trace Elemental Analysis, BAM Berlin, the buffer salts of analytical grade were purchased from Aldrich and Merck, and all the solvents were checked for fluorescent impurities. The analytical experiments were carried out in air-saturated solutions and, because of the pH-dependent complexation behaviour of $BP(OH)_2$ towards Zn^{II} and Cd^{II}, a phosphate buffer of physiological pH (pH 7.6) was used to keep the solutions at constant ionic strength.

Steady-state measurements were performed on a SPECORD M400/M500 spectrophotometer from Carl Zeiss Jena and a Perkin-Elmer LS50B fluorescence spectrometer. For the determination of the relative fluorescence quantum yields (Φ_f), the optical densities (o.d.) of the solutions at the excitation wavelengths were adjusted to an o.d. of 0.1 ± 0.001 in a 100 mm absorption cell. These solutions were then transferred to a 10 mm quartz cell and the fluorescence measurements were performed with a 90° standard geometry and an emission polarizer set at 54.7°. Quinine sulfate dihydrate (NIST standard reference material SRM 936) in 0.1 N H_2SO_4 (Φ_f=0.51 ± 0.03) was used as fluorescence standard [44,45]. For a typical analytical experiment, excitation and emission wavelengths were selected by monochromators with 2.5 nm (excitation) and 7.5 nm (emission) spectral bandwidth and the spectra were scanned at a speed of 50 nm min^{-1}. The time-resolved fluorescence measurements were either performed with synchrotron radiation from the Berlin Storage Ring for Synchrotron Radiation (BESSY) or with a laser impulse fluorometer with ps time resolution (ps-LIF) described elsewhere [24]. In the latter case, the second harmonic output of a regenerative mode-locked Ti : Sa laser at a repetition rate of either 82 or 4 MHz was used to excite the sample. In all cases, fluorescence was collected at right angles (monochromators with a spectral bandwidth of 8 nm). The excitation wavelength of the synchroton radiation was selected with a monochromator with 2 nm spectral bandwidth. The temporal response of the ps-LIF was 33 ps and the pulsed excitation source BESSY allowed for a temporal resolution of ca. 100 ps (single-bunch mode; 4.8 MHz). The fluorescence decay curves were recorded with a time-correlated single photon counting setup and a time division of 5.2 ps chn^{-1} (ps-LIF, 82 MHz version), 52.6 ps chn^{-1} (ps-LIF, 4 MHz version), and 55.5 ps chn^{-1} (BESSY) and typical count rates were in the order of $2–4 \times 10^3$ counts s^{-1} (ps-LIF) and 1×10^3 cps (BESSY). For the fluorescence decay measurements data were accumulated up to 20 000 (ps-LIF) or 5000 (BESSY) counts in the peak channel (CPC) for a single decay. But in order to have comparable total experiment times for the time-resolved and for the steady-state analytical measurements (see below) the CPC were generally lower in these experiments (results in Table 2). As the results of the time-resolved measurements presented in the analytical section of this paper are always based on the global analysis combining the data of 5 decays (with $\leq$4000 CPC per decay) or 20 decays (with $\leq$1000 CPC per decay), a sufficient level of statistical confidence is reached. The temporal calibration of the experimental setups for the

time-resolved measurements was checked with rose bengal in methanol ($\tau_f = 0.50 \pm 0.02$ ns; [46]), POPOP in ethanol ($\tau_f = 1.35 \pm 0.20$ ns; [47]), and fluorescein 27 in 0.1 N NaOH ($\tau_f = 4.50 \pm 0.03$ ns; [47]). For all the fluorescence measurements, emission polarizers were set at 54.7° and for the fluorescence excitation experiments, excitation polarizers were set at 0°. All the fluorescence spectra presented here are corrected for the spectral response of the detection system.

The steady-state emission spectra were analyzed with the software packages APROMAX and PARAMAX (U. Stahl/BAM) based on a linear singular value decomposition algorithm and for the analysis of the fluorescence decay data the software packages IBH Decay Analysis Software (IBH Consultants) and Global Analysis Software (Globals Unlimited) were employed. The data record analyzed consisted of 500 and 1024/2048 data points for the single steady-state spectrum and the single decay (BESSY/ps-LIF), respectively.

For all the steady-state analytical measurements, the data acquisition time was limited to intervals up to 15 min per spectrum and in the time-resolved analytical experiments the data were acquired up to 3 min per decay. In order to resolve the overlapping emission bands of $BP(OH)_2$ and its complexes, the emission wavelength-dependent decay curves were recorded from 420 to 500 nm in steps of 20 nm (measurement time of 15 min required) and from 410 to 600 nm in steps of 10 nm (measurement time of 1 h required).

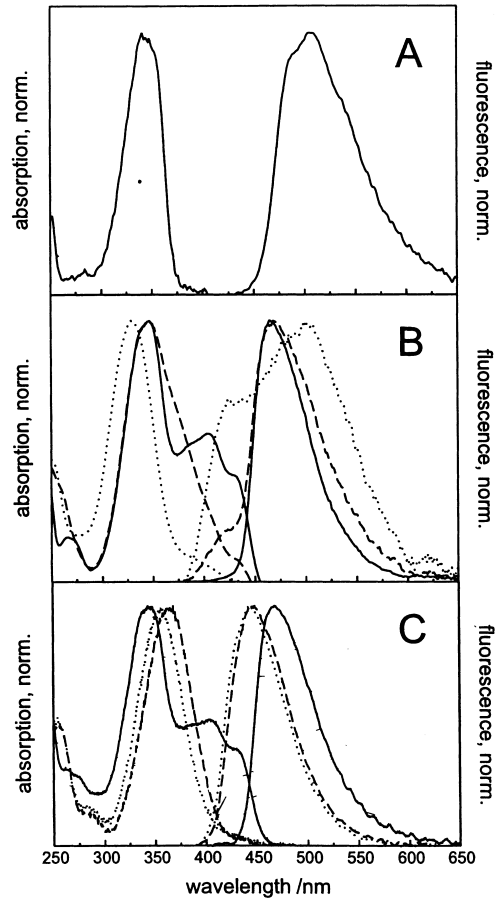

Fig. 1. Normalized steady-state spectra of $BP(OH)_2$ in acetonitrile (A; excitation at 340 nm), in aqueous solutions at different pH (B; (——— pH 4.8, – – pH 8.7, ····· pH 11.7; excitation at 330 nm), and of the Zn^{II} and Cd^{II} complex in water (C; ——— $BP(OH)_2$, – – Zn^{II} complex, ····· Cd^{II} complex; excitation at 350 nm).

3. Results

3.1. Steady-state absorption and emission behaviour

The steady-state absorption spectrum of $BP(OH)_2$ in water is broad, structureless and very different from the spectrum in protic and aprotic organic solvents and shows a strong pH dependence. On the contrary, the steady-state emission spectrum observed in water is only slightly blue-shifted compared to the emission spectra in organic solvents but maintains the bandshape. Only at pH values higher than ca. pH 7 a second emission band centered at 420 nm occurs. Fig. 1 combines the steady-state spectra of $BP(OH)_2$ in acetonitrile (part A), in aqueous solutions at different pH values (part B) and those of the Zn^{II} and the Cd^{II} complex in neat water (part C). The detailed analysis of the pH-dependent spectroscopic behaviour of $BP(OH)_2$ will be published in a separate paper [48].

At neutral pH, the absorption spectrum of $BP(OH)_2$ is composed of at least three subbands which originate in contributions of different keto and enol tautomers to the overall absorption band. Whereas in protic polar organic solvents only the dienol form is stable in the ground state, both tautomers are equally stable in aqueous solutions [40]. The four possible protonation sites and the strong tendency of the solvent to form intermolecular hydrogen bonds as well as the keto–enol tautomerism result in a complex multi-step reaction/equilibrium scheme. However, excitation at any wavelength between 320 and 450 nm yields the same emission spectrum and fluorescence quantum yield. Therefore, all the different ground state species should undergo a very fast proton transfer reaction upon excitation leading to the fluorescent zwitterionic diketo tautomer. This was experimentally confirmed by a comparative study of $BP(OH)_2$ and the corresponding 'ground state diketo tautomer', the 3′-hydroxy-1,1′-dimethyl-3-oxido-2,2′-bipyridinedium ion, carried out by Borowicz et al., who found a very different ground state but a very similar excited state behaviour for these two compounds [31].

Complexation to Zn^{II} and Cd^{II} leads to both a change in the absorption and the emission spectrum. The spectra of the 1 : 1 complexes (full complexation of the ligand) are included in Fig. 1. In both cases, sharp isosbestic points are observed in a UV–Vis-spectrophotometric titration experiment suggesting the formation of a well-defined equilibrium. However, a fit of the titration curves does not yield

Table 1
Spectroscopical properties of $BP(OH)_2$ and its Zn^{II} and Cd^{II} complexes in aqueous solutions

	Solvent	pH	λ_{abs}^{max} (nm)	λ_{em}^{max} (nm)	Φ_f	τ_f (ns)
$BP(OH)_2$	H_2O	6.4	345, (404, 430)[a]	467	0.040 ± 0.002	0.45 ± 0.02
$Zn^{II} \subset (BP(OH)_2$	H_2O	6.4	364	447	0.62 ± 0.02	6.17 ± 0.02
$Cd^{II} \subset BP(OH)_2$	H_2O	6.4	357	446	0.53 ± 0.02	5.24 ± 0.02
$BP(OH)_2$	Phosphate buffer	7.6	345, (404, 430)[a]	467	0.037 ± 0.002	0.55 ± 0.02
$Zn^{II} \subset BP(OH)_2$	Phosphate buffer	7.6	364	447	0.57 ± 0.02	7.26 ± 0.02
$Cd^{II} \subset BP(OH)_2$	Phosphate buffer	7.6	357	446	0.48 ± 0.02	6.22 ± 0.02

[a] Secondary maxima, see Fig. 1.

acceptable results for a pure 1 : 1 complexation but has to be described with the sum of a 1 : 1 and a 2 : 1 model. As would be expected for d^{10} metal ions, the spectral shifts observed upon complexation are small. The absorption band of the complexes fully overlaps with the absorption band of the free ligand and the emission bands are only shifted hypsochromically for ca. 20 nm. All the spectroscopic data of the three compounds are summarized in Table 1.

3.2. Time-resolved emission behaviour

The fluorescence of $BP(OH)_2$ in water of neutral pH decays monoexponentially with a decay time τ_f of 0.45 ns. This decay time varies with pH (ca. 0.7 ns below pH 2, ca. 0.2 ns above pH 10) and buffer environment and the data recorded in the phosphate-buffer employed yields $\tau_f = 0.55$ ns. When Zn^{II} or Cd^{II} ions are added to an aqueous solution of $BP(OH)_2$, a distinct second emitting species with a lifetime of 6.17 ns (for the Zn^{II} complex) or 5.24 ns (for the Cd^{II} complex) appears. The fluorescence decay curves in water are shown in Fig. 2 and the fluorescence lifetime data are included in Table 1.

When monitoring the fluorescence decays over the whole emission band of mixtures containing different concentrations of the free ligand and a certain cation, the decay curves could always be described by one (<420 nm, only emission

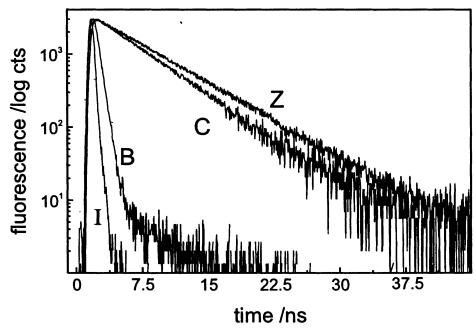

Fig. 2. The fluorescence decay profiles of $BP(OH)_2$ (curve B) and its Cd^{II} (curve C) and Zn^{II} (curve Z) complexes in water (I: instrumental response function of the experimental setup at BESSY; Traces of a long decay component with $\tau_f = 4.1 \pm 1.5$ ns and a relative amplitude of ca. 0.2% are often observed for uncomplexed $BP(OH)_2$, probably due to minor impurities in the buffer salts, acids, and bases used).

of the complex) or two exponentials and no rise time was detected at the red edge of the spectrum. This suggests that the complexes are stable in the excited singlet state and that no reaction takes place in the excited state on a time scale longer than 10 ps. Thus, ground state heterogeneity was assumed for the mathematical evaluation of the data.

3.3. Analytical measurements

Prior to the analytical measurements, a twenty point calibration curve for each ion and each experimental technique was recorded and yielded correlation coefficients of $r \geq 0.998$ in all cases. Then the samples containing unknown amounts of both metal ions were measured according to the following procedure:

- steady-state emission spectrum
- fluorescence decays at 5 different emission wavelengths ($\leq$4000 CPC; 15 min measurement time)
- fluorescence decays at 20 different emission wavelengths ($\leq$1000 CPC; 1 h measurement time)
- steady-state emission spectrum.

The second steady-state emission spectrum was recorded in order to exclude errors in the measurements due to photodecomposition of the sample.

For the analysis of a steady-state emission spectrum of a certain mixture of Cd^{II} and Zn^{II}, the spectrum was fitted with the spectra of the three single components employing a linear singular value decomposition algorithm and a measurement error of 1% was assumed. The results of six experiments are shown in Table 2 and two graphical examples for steady-state fits are given in Fig. 3.

In the upper part of Fig. 3 the measured curve (dotted line), the fit (full line) and the contributions of the single components to the fit (broken line) of sample #3 containing 3.59 mg l^{-1} Cd^{II} and 60 μg l^{-1} Zn^{II} (60-fold excess of Cd^{II} over Zn^{II}) are presented. A comparison of the plot and the data for sample #3 listed in Table 2 shows that the high excess of Cd^{II} over Zn^{II} is overrated in the fit although the fit describes the measured spectrum well. The contributions of the components associated with the emission of free $BP(OH)_2$, the Zn^{II}, and the Cd^{II} complex are labeled B, Z, and C, respectively. The same effect is still observed for a five-fold excess of Cd^{II} over Zn^{II} in sample #6 (lower part of

Table 2
Results of the determination of Zn^{II} and Cd^{II} in aqueous solutions with $BP(OH)_2$ and steady-state (SSF) or time-resolved (TRF) fluorometry

Sample	C/Cd^{II} (mg l^{-1})				c/Zn^{II} (mg l^{-1})			
	Given	Found SSF	Found TRF[a]	Found TRF[b]	Given	Found SSF	Found TRF[a]	Found TRF[b]
#1	1.45	2.56	1.40	1.42	0.26	0.13	0.25	0.25
#2	0.94	2.84	0.92	0.93	0.52	0.31	0.53	0.52
#3	3.59	4.67	3.65	3.54	0.06	0.01	0.02	0.04
#4	0.75	0.50	0.74	0.75	0	0.02	0.006	0.001
#5	0.20	1.18	0.10	0.23	0.23	0.16	0.24	0.23
#6	3.59	6.05	3.50	3.60	0.71	0.33	0.74	0.70

[a] Five decays from 420 to 500 nm in steps of 20 nm analyzed.
[b] 20 decays from 410 to 600 nm in steps of 10 nm analyzed.

Fig. 3). Both examples illustrate the poor correlation between a goodness of the fit and the accuracy of the analytical result for the steady-state technique.

In the case of the time-resolved measurements, the single decays were fitted to three fixed exponentials performing ten trial fits with different initial estimates for the amplitudes in order to exclude optimizations into local minima. The wavelength-resolved recorded decay curves were then analyzed globally [49,50]. Taking into account the ground state heterogeneity, global analysis allows the construction of the decay associated spectra (DAS (λ, i)) of the single emitting species according to Eqs. (1) and (2) [49]

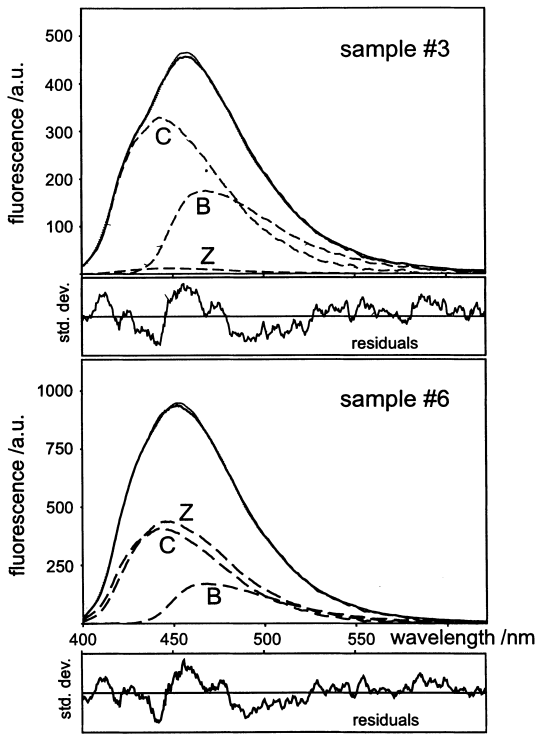

Fig. 3. Fitting results obtained by analyzing the steady-state emission spectra of the samples #3 and #6 (——— measured spectra, ····· fits, – – single components attributed to uncomplexed $BP(OH)_2$ (curve B), its Cd^{II} (curve C) and Zn^{II} (curve Z) complexes).

$$F(\lambda, t) = \sum_i a_i(\lambda) e^{-t/\tau_i} \quad (1)$$

$$\mathrm{DAS}\,(\lambda, i) = \frac{a_i(\lambda)\tau_i F^{SS}(\lambda)}{\sum_i a_i(\lambda)\tau_i} \quad (2)$$

where $i = 1$ denotes component 1 ($\tau_f = 0.55$ ns) attributed to $BP(OH)_2$, $i = 2$ denotes component 2 ($\tau_f = 7.26$ ns) attributed to the Zn^{II} complex, and $i = 3$ denotes component 3 ($\tau_f = 6.22$ ns) attributed to the Cd^{II} complex and F^{SS} (λ) is the measured steady-state emission spectrum. Within the global analysis algorithm, the decay times of the species are fixed (i.e., values obtained for the single components in pure solutions, s. Table 1) and linked for the analysis of all the decays while the program varies the pre-exponential factors until the changes in the error surface (χ^2 surface) are minimal, i.e., convergence is reached. During every iteration (i) the fit is judged for every single decay (local χR^2) and (ii) all the fits are judged globally (global χR^2). The errors for all the measurements presented here were below global $\chi R^2 = 1.5$. The results of the determination of Zn^{II} and Cd^{II} with time-resolved fluorometry (decays collected at 5 emission wavelengths; 15 min measurement time) are included in Table 2 and the resulting decay associated spectra of sample #5 is shown in Fig. 4.

4. Discussion

Comparison of the analytical results shown in Table 2 clearly reveals the improvement in the determination of chemically very similar compounds, e.g., the complexes of Zn^{II} and Cd^{II} with $BP(OH)_2$, when going from steady-state to time-resolved fluorometry. Whereas the mean deviation from the true value for all the single concentrations equals 105% in the case of steady-state fluorometry, the use of time-resolved techniques leads to considerably smaller deviations, 13% (for the 5-emission wavelength measurements) and 6% (for the 20-emission wavelength measurements), respectively. When recording a time- and wavelength-resolved emission matrix of a sample, even the detection of a relatively small amount of Zn^{II} besides a large amount of Cd^{II} (see Table 2, sample #3) and the

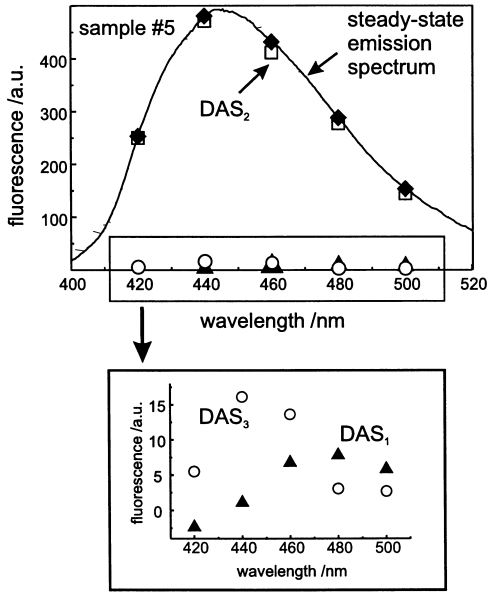

Fig. 4. Fitting results obtained by globally analyzing five decays of sample #5. The measured steady-state spectrum (———) and the spectral contributions (DAS_i) of the single species are displayed (▲ DAS_1 ($\tau_f = 0.55$ ns, $BP(OH)_2$), □ DAS_2 ($\tau_f = 7.26$ ns, Zn^{II} complex), ○ DAS_3 ($\tau_f = 6.22$ ns, Cd^{II} complex)). For a better representation of DAS_1 and DAS_3, the region of weak fluorescence is enlarged in the lower part of Fig. 4. The black rhombs (◆) in the upper part denote the calculated steady-state spectrum at the emission wavelengths employed in the time-resolved measurements of sample #5 ($F^{SS}_{calc}(\lambda) = \sum_i DAS(\lambda, i)$ for $\lambda = 420$, 440, 460, 480, and 500 nm).

quantification of Cd^{II} in solutions containing both ions at nearly the same concentration (see Table 2 and Fig. 4, sample #5) is possible. The apparent mismatch between the spectral contributions of the single complexes in Fig. 4 ($DAS_2 \gg DAS_3$) and the concentrations of Zn^{II} and Cd^{II} in sample #5 given in Table 2 ($c_{Zn} \cong c_{Cd}$) is due to the higher complex stability constant ($\log K$[1]: 4.1 for Zn^{II}; 3.4 for Cd^{II}) and fluorescence quantum yield (0.62 for $Zn^{II} \subset (BP(OH)_2$; 0.53 for $Cd^{II} \subset BP(OH)_2$) of the Zn^{II} complex. Although the fitting of the steady-state data always yielded small errors in terms of describing the shape of a spectrum, the analytical validity of the fits is rather poor. The strongly overlapping emission bands of all the three compounds and especially the two complexes exclude the application of steady-state spectroscopy in this field of analytics. For the same temporal expense, i.e., a measurement time of 15 min, the employment of time-resolved emission spectroscopy at five different emission wavelengths leads to an improvement in the validity of the analytical result of nearly one-order of magnitude. This is mainly based on the increased precision of the measured fluorescence lifetime ($<\tau_f> = 6.7$ ns, temporal resolution ≤ 0.05 ns) compared with the spectral resolution of the steady-state emission measurements. Employing time-resolved fluorometry we were able to detect Zn^{II} (Cd^{II}) accompanied by an up to five-fold (two-fold) molar excess of Cd^{II} (Zn^{II}). A further increase in sensitivity is achievable when decreasing the step size in the wavelength-resolved recording of fluorescence decays but aiming at an on-line and in-situ application of this technique the prolongation of both the time for the analytical measurement and for the data evaluation is unfavourable. Depending on the photostability of the fluorescent probe employed and the complex formed as well as the sensitivity and capability of the detection system, higher count rates due to higher excitation intensities could lead to an increase in CPC and thus an additional increase in sensitivity. This is especially possible with the high excitation rates used (ps-LIF in the 4 MHz mode or BESSY in the 4.8 MHz single bunch mode) which exceed the observed count rates (≤ 10 kHz) by orders of magnitude. For this system, due to the differences in higher complex stability constants ($\log \beta_{tot,\ Zn} > \log K$) and fluorescence quantum yields ($\Phi_{f,\ Zn} > \Phi_{f,\ Cd}$), the determination of Cd^{II} in the presence of Zn^{II} will always be less sensitive than vice versa. An analytically more favourable discrimination between both ions spanning for example three or more orders of magnitude in a simultaneous determination is not possible due to the relatively small temporal difference between the fluorescence lifetimes of the two complexes ($\Delta\tau_f = 1$ ns).

5. Conclusions

Aiming at the simultaneous determination of different but closely related metal ions in liquid aqueous media under in situ conditions we have shown the improvements when employing not only the spectral but the temporal evaluation of emission data obtained for a mixture of components. Many fluorescent probes such as $BP(OH)_2$ form stable complexes with different metal ions which are spectrally largely overlapping. In this case, the one-dimensional evaluation of spectral emission data mostly yields analytically unsatisfactory results, and a discrimination between three or more emitting species in a mixture is not possible. However, in the course of the progress in laser technology, low-cost pulsed excitation sources emitting in the Vis–NIR region of the spectrum are commercially available and allow the measurement of the fluorescence lifetime as an additional dimension in a comparably short time. For $BP(OH)_2$, collection of this additional information gained an increase in analytical accuracy of nearly one-order of magnitude. However, further research has to be focused on the spectral and lifetime tailoring of fluorescent probes in order to show more pronounced complexation-induced differences in fluorescence lifetime upon binding to various metal cations.

Acknowledgements

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[1]Complex stability constant in water for M+L→ML.

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