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An Automated Approach to Luminescence Lifetime and Intensity Titrations

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An apparatus is described for the automated collection of luminescence emission decay curves over a wide range of analyte concentrations. The decay curves allow for determination of the excited-state lifetime or calculated steady-state intensity of a luminophore as a function of the analyte concentration. The data presented here demonstrate the use of the apparatus for pH titrations.

KEY WORDS: Lifetime measurement; titration; automation; fluorescence-based probes; luminescence-based probes.

INTRODUCTION

Luminescent probes are used extensively as sensors for a variety of analytes, including H^+ [1–6], CO_2 [5,7], K^+ [3], Ca^{2+} [3,8–11], and other metal ions [12,13]. An important step in the development of new probe species is the characterization of the luminescence response to changes in analyte concentration. In our laboratory, we have produced several species as potential pH probes and routinely measure the lifetime and intensity of these species at small incremental pH changes (~0.25 to 0.5 pH unit) over a wide (2–12) pH range. In total, the analysis requires dozens of measurements, which can often take a day or more to acquire. For this reason, we have developed an apparatus for automated lifetime titrations, which reduces the time required to acquire a complete data set to approximately 3 h, with minimal operator effort. We have used the apparatus for pH titrations; however, it could be used with slight modification for titrations of metal ions or other analytes.

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EXPERIMENTAL

The computer, which serves as the hub of the apparatus, is interfaced with a Tektronix TDS 540 digital oscilloscope via an IEEE card and uses a Data Translation DT2801 data acquisition card as the interface for other peripherals, as shown in Fig. 1. The acquisition card receives analog data from a Corning 340 digital pH meter and also controls an optically isolated 120-V digital switch. The optically isolated 110-V AC relay is used to toggle a Sage Instruments Model 341 syringe pump on and off. The optical switch was required to turn the pump on and off because the pump was not designed for computer interfacing. All software was written using Labview 4.0 [14] including several drivers provided by Data Translation.

The pH meter is operated in Continuous Read mode and is connected to the data acquisition card through its strip-chart terminal output. This output produces a voltage that is proportional to the pH and, after initial calibration, can be interpreted by the Labview program as a direct and continuous pH measurement. The program reads the pH and compares it to preprogrammed pH checkpoints. If the pH is lower than the next programmed pH value, a signal is sent to the optical switch, which toggles the power to the syringe pump. The pump delivers titrant to the sample container through a Teflon tube. The pH is

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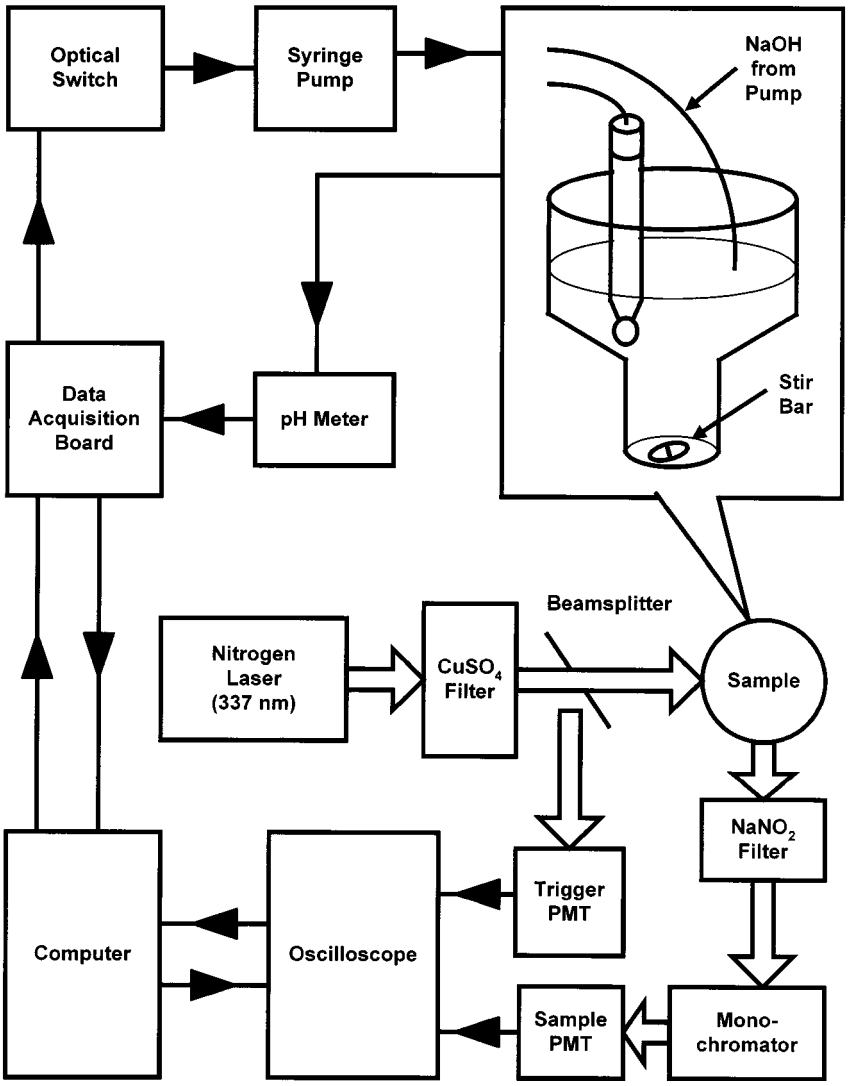

Fig. 1. Schematic showing the computer-controlled lifetime titration apparatus. Filled arrows indicate the flow of information, and open arrows indicate light paths.

monitored until the pH checkpoint is reached, at which time the power to the pump is turned off. Thirty seconds is allowed for thorough mixing, the pH is recorded, and the emission decay curve is measured. Generally the equilibrated pH is within 0.05 pH unit of the programmed value.

The emission decays were recorded using a Laser Science Inc. VSL-337 pulsed nitrogen laser, operating at approximately 10 Hz, and a Tektronix TDS 540 digital oscilloscope. The laser pulse is directed through a cuvette containing aqueous $CuSO_4$ to remove long wavelength components, and a microscope slide is used as a beamsplitter to send a portion of the laser pulse to a PMT, which triggers the oscilloscope. The sample emission passes through a cuvette containing aqueous $NaNO_2$ to remove scattered 337-nm laser light and a monochromator to a second PMT, which is connected to the oscilloscope. The oscilloscope records the decay traces with the first 20% of the data points in the pretrigger time interval to establish a well-defined baseline. After recording several hundred decay traces, the oscilloscope exports the average trace to the PC. The PC then writes a text file, which contains the *x* and *y* values of the emission decay, and uses the current pH value as the file name. A representative decay trace and portions of the data file are shown in Fig. 2.

The process of monitoring the pH, toggling the syringe pump, and recording emission decays is continued until the end of the specified pH range is reached. During the process, the operator must make periodic man-

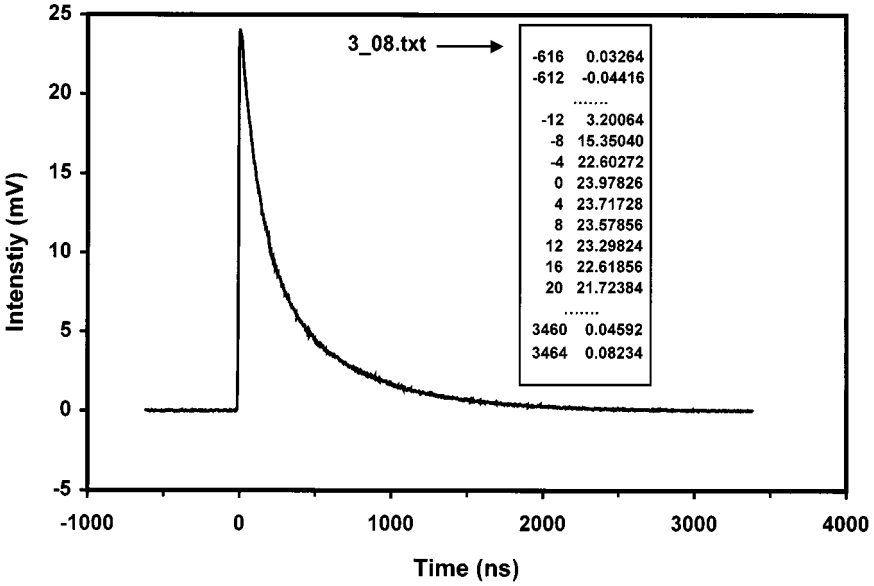

Fig. 2. An averaged ($n \cong 250$) emission trace collected by the oscilloscope at pH 3.08. The inset shows portions (pretrigger, at trigger, and long after trigger) of the data file saved with the filename "3__08.txt." The data in the first column are time coordinates, and those in the second column are the corresponding intensities.

ual adjustments to the delivery rate of the syringe pump to maintain a rate that is appropriate for the pH region. Because no buffers have been added, very slow delivery rates are required near pH 7. A programmable syringe pump would allow the process to run unattended.

The custom sample compartment is constructed from Pyrex and has a large reservoir to accommodate the change in volume that occurs during the titration. The lower portion has a diameter of 2.5 cm and the upper portion has a diameter of 6 cm.

Rhenium complexes were synthesized by a previously published procedure [15] and were determined to be pure by thin-layer chromatography and by the method of relative excitation spectra [15,16]. There was no evidence of photodecomposition for any of the complexes under the conditions employed for these measurements. For all samples, two stock solutions were prepared. A 25-ml sample acidified to ~pH 2.0 was placed in the Pyrex sample container (see Fig. 1), and a 50-ml sample at ~pH 12.5 was placed in the syringe. The concentration of the luminophore was approximately 50 μM and was the same in both acidic and basic solutions. As the basic solution is titrated into the acidic solution, the pH increases, while the concentration of the luminophore remains constant. Organic solvents were added to some samples (up to 20% ethanol or acetonitrile) to ensure solubility of the complexes over the pH range studied. However, this requirement was specific for these samples and is not generally required by the apparatus.

Following the recording of emission data, each trace was fit to a multiexponential decay equation using a nonlinear least-squares fitting algorithm. The generalized equation is given by Eq. (1).

$$I(t) = \sum_{i=1}^{n} \alpha_i e^{-t/\tau_i} \tag{1}$$

where $I(t)$ is the emission intensity as a function of time, and α_i and τ_i are the initial intensity and lifetime of component i, respectively. The fitting program gives as its output one, two, or three pairs of α_i and τ_i variables to describe each decay. A single-exponential decay ($n = 1$) was adequate for most species; however, multiple exponentials were required in some cases for which the mean lifetime (τ_m) and preexponential-weighted lifetime (τ_{PE}) were calculated from Eqs. (2) and (3).

$$\tau_m = \frac{\sum_{i=1}^{n} \alpha_i \tau_i^2}{\sum_{i=1}^{n} \alpha_i \tau_i} \tag{2}$$

$$\tau_{PE} = \frac{\sum_{i=1}^{n} \alpha_i \tau_i}{\sum_{i=1}^{n} \alpha_i} \tag{3}$$

The integrated intensity for each emitting component is given by the product of the lifetime and the preexponential

factor, and therefore, the total integrated intensity for any emission trace can be calculated from Eq. (4).

$$I(\text{total}) = \sum_{i=1}^{n} \alpha_i \tau_i \quad (4)$$

These intensities are equivalent to those that would be obtained from a standard steady-state measurement on a conventional fluorimeter, although they may be less precise due to fluctuations in the light source.

Titration curves were fit to a standard two-component model. Details have been provided previously [17].

RESULTS

Figures 3 and 4 show the lifetime and intensity data for two rhenium complexes in mixed solvents obtained using the apparatus described here. In Fig. 3, the preexponential-weighted lifetime of $Re(CO)_3$(dmambpy)Cl, (dmambpy is 4,4′-bis-dimethylaminomethyl-2,2′-bipyridine) is plotted as a function of pH in the range 2 to 12. The titration curve produced by the data shows a clear and significant change in excited-state lifetime occurring between pH 4 and pH 7, indicating that this species may serve as a useful lifetime-based pH probe in this region. The $[Re(CO)_3(phen)(py\text{-}3\text{-}OH)]ClO_4$ (phen = 1,10-phenanthroline, and py-3-OH = 3-hydroxypyridine) complex exhibits very little change in lifetime but shows a dramatic change in intensity, as calculated by Eq. (4), over the pH range 6–8, with an effective pK_a of 7.1 as shown in Fig. 4. The sets of emission traces used to compile Figs. 3 and 4 were each collected in approximately 3 h.

Figure 5, showing the measured peak intensity of $[Re(CO)_3(\phi_2phen)(py\text{-}3\text{-}OH)]ClO_4$($\phi_2$phen = 4,7-diphenyl-1,10-phenanthroline) in 20% ethanol, as determined by a manual titration, is included for comparison. The data set shown in Fig. 5 was obtained by manual titration, followed by intensity measurements using a SPEX Fluorolog 1680 spectrofluoimeter. This complex has pH-dependent photophysical properties which are very similar to those of the $[Re(CO)_3(phen)(py\text{-}3\text{-}OH)]ClO_4$ complex shown in Fig. 4. Although the data set acquired by manual titration (Fig. 5) is clearly more sparse than that acquired by the automated titrator (Fig. 4), the time required for the collection of the data by manual titration was actually greater.

CONCLUSIONS

This technique has allowed us to acquire a large number of emission decay traces in a very short time. More important is the fact that the very slow injection rate of the syringe pump allows for a gradual pH change that was difficult or impossible by manual titration techniques. It was particularly difficult in the pH range 5 to 9, where the most important data are collected. The gradual change in pH allows for the collection of several decay

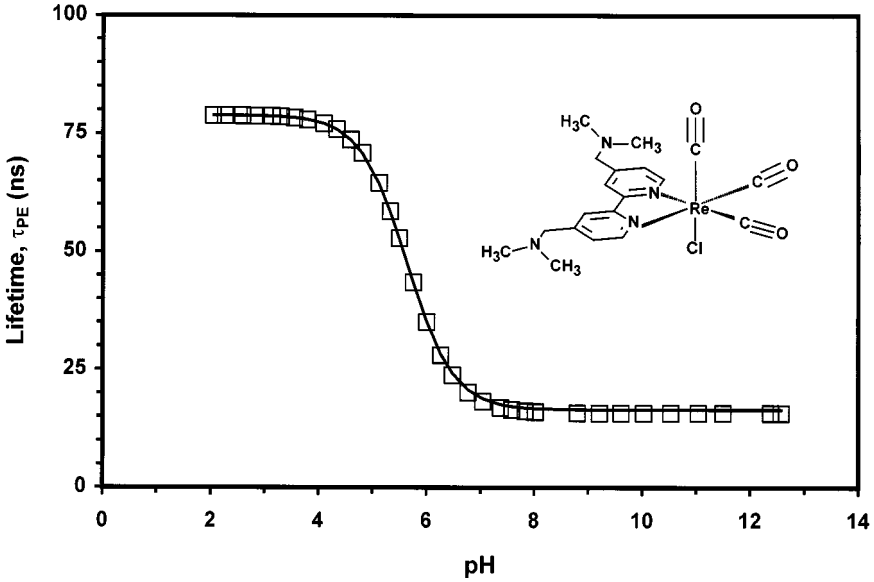

Fig. 3. pH titration curve constructed for $Re(CO)_3$(dmambpy)Cl in 20% MeCN. The plot shows the preexponential-weighted lifetime as a function of pH. The solid line is the best fit to a standard titration curve mode.[17].

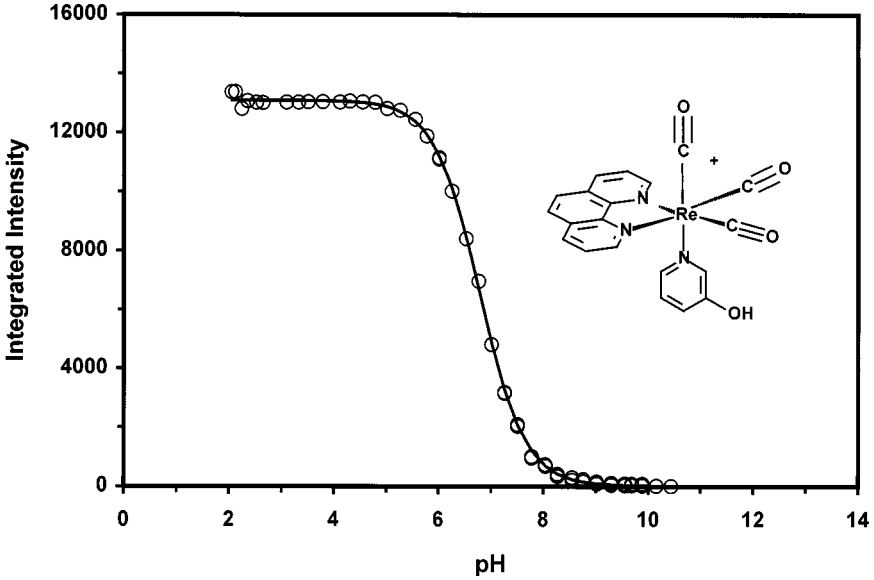

Fig. 4. pH titration curve for $[Re(CO)_3(phen)(py\text{-}3\text{-}OH)]ClO_4$ in 20% ethanol. The plot shows the integrated intensity as a function of pH. The solid line is the best fit to a standard titration curve model.[17].

traces at evenly spaced intervals during the titration, thus providing a more useful data set. The data compiled allowed for a very accurate determination of the necessary parameters to characterize the pH-dependent luminescent properties of the complexes under investigation. Although syringe pumps with computer interface capabilities are commercially available, we know of no existing titration apparatus which collects emission decay data.

We have found the new automatic titration system to be a dramatic improvement over the manual method. Not only are data collected more quickly, but the higher level of control afforded by the syringe pump allows for

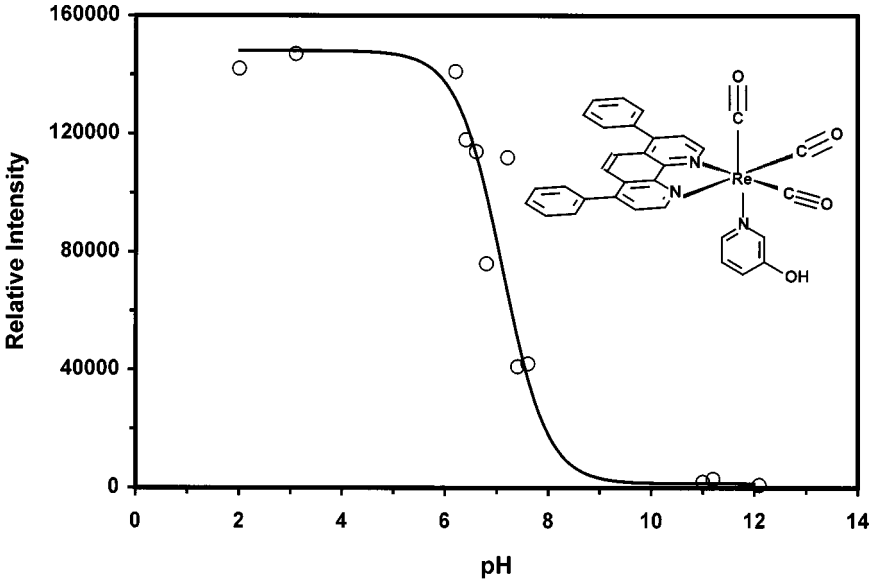

Fig. 5. pH titration curve for $[Re(CO)_3(\phi_2phen)(py\text{-}3\text{-}OH)]ClO_4$ in 20% ethanol. The plot shows the peak emission intensities as a function of pH as determined by a manual titration. The solid line is the best fit to a standard titration curve model.

more data to be collected in the most important pH regions. This apparatus can be easily modified for titrations of other analytes.

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