

Room-Temperature Phosphorescence (RTP) in Aqueous Solutions. An Advanced Undergraduate Laboratory Experiment

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Abstract: This paper presents a detailed description of the steps and conditions required to obtain room-temperature phosphorescence signals in aqueous solution. A complete laboratory experiment, designed to familiarize students with the uses of this type of spectroscopic signal and, at the same time, the application of computational methods (model fitting and parameter estimation) to obtain relevant thermodynamic results, is described. The experiment involves the measurement of the phosphorescence spectra of acenaphthene (used as a model system) at room temperature through the formation of inclusion complexes with β - and γ -cyclodextrins, the determination of the inclusion-constant value for the γ -cyclodextrin complex, and the measurement of phosphorescence lifetimes. The presence of 2,3-dibromopropanol with heavy atoms is necessary in order to obtain the RTP emission. Deoxygenation with sodium sulfite is necessary in the case of β -cyclodextrin, but not in the case of γ -cyclodextrin. A comparison between the phosphorescence intensity obtained in the presence of cyclodextrins with that from a system without cyclodextrin in a concentrated solution of potassium iodide deoxygenated with sodium sulfite is also presented and discussed.

Simple phosphorescence experiments on a solid surface have been previously described for undergraduate students [1]; however, in view of recent advances, such as the development of new analytical phosphorescence approaches in solution, the advent of commercial instruments containing pulsed sources with gated detectors, and the availability of microcomputers in routine laboratories, we have considered it timely to introduce this topic in the chemistry curriculum through a laboratory experiment.

With the purpose of aiding students in their understanding of the different aspects of this experiment, both theoretical and practical considerations are briefly described to them prior to conducting the experiment.

Phosphorescence Emission. Photoluminescence is defined as an emission by atoms or molecules from an excited energy level that has been produced by absorption of incident radiation. Photoluminescence involves two emission processes: fluorescence and phosphorescence. The difference between these radiations lies in the nature of the excited state from which the emission occurs: fluorescence is the photon emission that results from a singlet excited state, and phosphorescence is the emission resulting from a triplet excited state. Because the ground state of a neutral molecule is usually a singlet state, it can be concluded that fluorescence (which involves a singlet-singlet transition) is quantum mechanically allowed, but phosphorescence (which involves a transition between states of different multiplicity) is not allowed. Due to this latter fact, phosphorescence processes have a low probability of occurrence. Their emissive rates are low and, therefore, phosphorescence lifetimes are longer than those corresponding to fluorescence lifetimes (see below) [2]. Figure 1 shows a schematic energy-level diagram for these processes. In this diagram, the lowest horizontal line represents the ground-state energy level (S_0). The upper lines, S_1 and T_1 , represent the first

singlet and triplet excited states, respectively. Generally, the energy of S_1 is higher than that of T_1 ; thus, the phosphorescence Stokes shifts (difference between the wavelength emission and the wavelength absorption) are larger than those corresponding to fluorescence processes. In the figure, both fluorescence and phosphorescence emissions are shown as straight arrows, and the nonradiative decay processes are indicated as wavy arrows. The triplet states of molecules are mainly deactivated by efficient nonradiative processes; therefore, phosphorescence emission is usually observed only at low temperatures (liquid nitrogen) in viscous media or from molecules adsorbed on solid surfaces where these nonradiative processes are minimized or deactivated. New advances in analytical phosphorimetry, however, have shown that phosphorescence emissions can be also obtained at room temperature in solution. It was demonstrated that several analytes are able to give room-temperature phosphorescence (RTP) in organized media such as micelles and cyclodextrin solutions [3–5]. Cyclodextrins (CDs) are cyclic oligosaccharides composed of D-glucose residues obtained by $\alpha(1\rightarrow4)$ linkages. The three major cyclodextrins, α , β , and γ -CDs, are formed by six, seven, and eight glucopyranose units, respectively. Several important characteristics of the CDs are summarized in Table 1, and Figure 2 shows a schematic representation of β -CD. Due to the conical shape of the CD cavity, these molecules have the ability to form host-guest complexes with different compounds of adequate size and polarity [6]. The function of the CD is to offer a shielding environment to the excited species from quenchers and nonradiative pathways [7].

The presence of a heavy atom is an important factor for RTP detection because this type of atom favors the intersystem crossing from the singlet state to the triplet state of the guest.

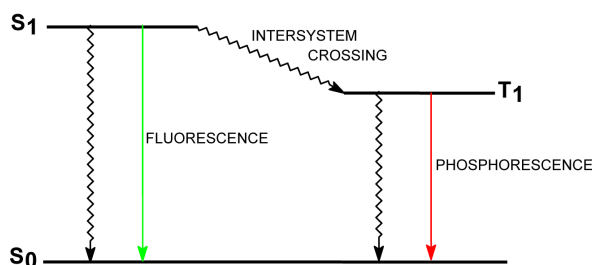


Figure 1. Partial energy diagram for photoluminescence processes. The straight and wavy arrows indicate luminescence and radiationless processes, respectively. S_0 , S_1 , and T_1 represent the singlet ground-state, the first singlet excited-state, and the first triplet excited-state, respectively.

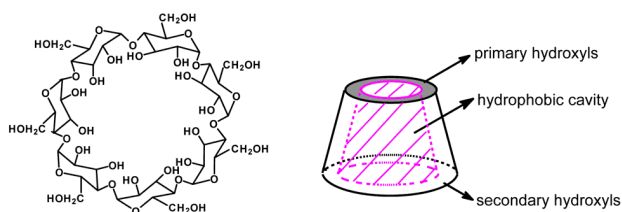


Figure 2. Structure of β -CD.

Table 1. Physical Parameters of Cyclodextrins^a

	α -CD	β -CD	γ -CD
Molecular weight	972.8	1135	1297
Cavity diameter (\AA)	6.0	8.0	10.0
Cavity volume (\AA^3)	176	346	510
Water solubility (g per 100 mL)	14.5	1.85	23.3

^aRef 6

The heavy-atom effect is caused by spin-orbit coupling, which allows for relaxation of spin selection rules. If the substrate does not bear a heavy atom, the latter must be added externally. In this case, the formation of a ternary complex formed among the original analyte, the CD, and the additional third component containing the heavy atom produces adequate protection from quenching and constitutes a feasible approach for enhancing the phosphorescence emission in solution. For this to occur, both the lumiphor and the heavy atom need to be in close proximity at the same time, that is, inside the cyclodextrin cavity, to allow sufficient interaction between the analyte and the heavy atom compound to produce effective population of the triplet state of the analyte.

Another important factor for RTP detection is the absence of dissolved oxygen in the working solutions because this molecule is an efficient phosphorescence quencher [8]. Different modes of deoxygenation can be used, such as nitrogen bubbling or sodium sulfite addition. In this latter case, oxygen is consumed during the oxidation of sulfite to sulfate [9]; however, it should be noted that in several systems the inclusion complex formation produces an efficient protection of the triplet state to the extent that it becomes possible to work in aerated samples [10, 11]. This is a particular advantage of using cyclodextrins as opposed to the use of micelles because in micellar media deoxygenation is always necessary.

Recently, a new way for obtaining RTP in solution without the use of an organized medium was proposed [12]. In this case, the RTP emission is a consequence of intermolecular protection when analytes are in the presence of a significant amount of a heavy-atom salt and sodium sulfite as oxygen scavenger. The salts more often used are potassium iodide and thallium(I) nitrate. This latter type of phosphorescence emission has been named heavy-atom-induced RTP (HAI-RTP), and is also described in the present work.

Phosphorescence Lifetime. The phosphorescence lifetime of a molecule represents the average amount of time this molecule remains in the excited triplet state prior to its return to the ground state. Knowledge of lifetimes is useful for the characterization of mixtures of phosphorescent analytes and for the calculation of rate constants of triplet states [13]. Ideally, phosphorescence intensity decays after the removal of the exciting source with first-order kinetics; thus, the general equation relating the phosphorescence intensity and the lifetime is [2]

$$I = I_0 e^{-t/\tau} \quad (1)$$

where I is the phosphorescence intensity at time t , I_0 is the maximum phosphorescence intensity during excitation, and τ is the average lifetime of the triplet excited state. Due to the usually large lifetimes of excited triplets, measurements are easier than those corresponding to the decay of fluorescence. By means of an exponential adjustment of the decay curve obtained for a selected system, the corresponding value of τ can be calculated.

Selected Systems. As examples of phosphorescent systems, acenaphthene (AN) in aqueous solution and in the presence of either β -CD or γ -CD was selected. AN (Figure 3) is an organic molecule belonging to the polyaromatic hydrocarbon (PAH) group. Evidence for the existence of a 1:1 complex formed between AN and either of the two cyclodextrins studied here were obtained in previous works [14, 15]. Formation constants of 130 and 120 M^{-1} for AN- β -CD and AN- γ -CD binary inclusion complexes were obtained, respectively. Further, RTP emission from ternary complexes formed between AN, the corresponding CD, and a bromoalcohol (2,3-dibromopropanol) was observed [15, 16]. The RTP signal from AN was also obtained in the presence of potassium iodide and sodium sulfite [17]. Although in the latter case thallium(I) nitrate can also be employed as the heavy-atom source, its use is not recommended in undergraduate laboratories because of its high toxicity.

Time Requirements. The whole experiment is designed to be carried out in approximately 3.5 hours; however, because it is presented in three separate modules, it can be performed in sequential laboratory sessions. The timeline is outlined below.

Preparation of solutions (30 laboratory minutes)

- Module I: Spectrophosphorimetric spectra acquisition (30 laboratory minutes)
- Module II: Decay curves acquisition (30 laboratory minutes)
- Module III: Inclusion constant determination (1 laboratory hour)
- Total computational work (1 hour)

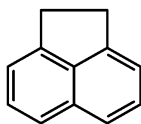


Figure 3. Chemical structure of acenaphthene (AN).

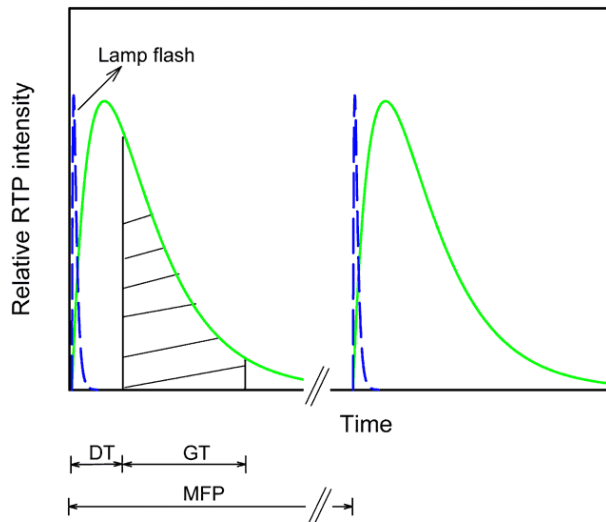


Figure 4. Schematic diagram of events occurring during the cycle of sample excitation and observation in a pulsed-source phosphorimeter system. DT = delay time, GT = gate time, MFP = minimum flash period.

Table 2. Instrumental Conditions for the Decay-Curve Determinations^a

	Cyclodextrin systems	Iodide system
Lower limit ^b	0 ms	50 μ s
Upper limit ^b	200 ms	1000 μ s
Resolution ^c	5 ms	50 μ s
Repetitions	100	100

^aThe excitation and emission wavelengths, the slit widths, the delay and gate times, and the photomultiplier sensitivity are those indicated in the text. ^bThe lower and upper limits indicate the range of times recorded. ^cThe resolution denotes the difference (in units of time) between successive points of the decay curve.

Experimental Procedure

Materials and Solutions. Acenaphthene and 2,3-dibromopropanol were purchased from Aldrich (Milwaukee, WI, USA), and methanol, potassium iodide, and sodium sulfite were obtained from Merck (Darmstadt, Germany). The β - and γ -cyclodextrins were acquired from Cyclolab (Cyclodextrin Research and Development Laboratory, Budapest, Hungary). All reagents were high-purity grade and used as received. Aqueous solutions were made with double-distilled water. In order to obtain signals of good analytical quality, the working conditions, such as the optimum reagent concentrations in the experimental solutions, must be carefully selected before conducting each RTP experiment. In many systems this is a critical step for the development of the RTP emission. In recent years, experimental design, such as response-surface methodology, has been conveniently applied in order to find the best working requirements [18]. For time reasons in the

present work the conditions for the systems to be used are suggested to the students. A 2.00×10^{-2} M stock solution of AN was prepared in methanol. An 1.00×10^{-4} M aqueous solution was made by placing the appropriate aliquot of the stock solution (0.5 mL) in a 100.0-mL volumetric flask, evaporating the methanol by a stream of dry nitrogen, and diluting with water. Aqueous solutions of 0.010 M β -CD, 0.04 M γ -CD, and 0.4 M sodium sulfite were prepared by weighing the required amount of each compound and diluting with water. In the experiment involving KI, the solid reagent was directly weighed in order to obtain the final 2 M solution (3.32 g added to a 10-mL volumetric flask and diluted to the mark).

Apparatus. RTP signals are easily obtained in the absence of fluorescence and with a minimum of scattered radiation from a luminescence spectrometer equipped with a pulsed-source gated detector. The sequence of events with a pulsed-source gated detector system is as follows [19, 20]. After an initial pulse of a xenon flash lamp (the source of energy) with a duration of approximately 13 μ s or less, the phosphorescence intensity climbs to a maximum intensity and then decays exponentially. After a delay time (DT) when the source flash has decayed, the detector is turned on and the phosphorescence intensity is integrated and measured. This latter time is named gate time (GT). The detector system is then turned off and the overall sequence repeated. Figure 4 shows a typical decay curve with the times mentioned above.

It is necessary to point out that the use of a commercial instrument containing a pulsed source with a gated detector is very convenient for these experiments although is not mandatory. Alternatively, an older luminescence instrument with a chopper system can be used.

In the present work, phosphorescence experiments were done on an Aminco Bowman Series 2 luminescence spectrometer equipped with a 7-W pulsed xenon lamp, a Pentium personal computer, a GPIB (IEEE-488) interface card for computer instrument communication, and a thermostated cell holder. Data acquisition and data analysis were performed by use of the AB2 Series 2 software running under the Windows 98 operating system. The measurements were carried out using 1.00-cm quartz cells and slit widths of 16 nm. All solutions were excited at 286 nm. The signals were obtained by measuring the emission at 520 nm. In the cyclodextrin systems, the delay time and the gate time were kept at 500 and 2000 μ s, respectively, and in the iodide system, they were set at 100 and 400 μ s, respectively. In all cases, the detector voltage was 1000 V.

AN phosphorescence spectra. With the purpose of obtaining the excitation and emission maxima for the RTP of AN, the following procedure was applied. An aqueous solution containing 1×10^{-5} M AN, 0.008 M β -CD, 0.4% (v/v) DBP, and 0.04 M sodium sulfite was prepared from the concentrated standard solutions. The emission spectrum of this solution was recorded using a λ_{exc} of 286 nm, which is the excitation maximum corresponding to the fluorescence emission. The RTP spectrum obtained shows two maxima at 484 and 520 nm and a shoulder at 559 nm. The excitation spectrum was then measured by fixing the emission monochromator at 520 nm, and a maximum in the RTP at $\lambda_{\text{ex}} = 286$ nm was detected. Thus, we can consider that λ_{ex} equal to 286 nm and λ_{em} equal to 520 nm are the maxima for excitation and emission, respectively. A similar procedure was applied for both the γ -CD system and the unprotected mixture of AN and potassium

Table 3. Spreadsheet with the Protocol for the Inclusion-Constant Determination^a

Filenames ^b	1×10^{-4} M AN volume (mL)	2,3-DBP volume (μ L)	4×10^{-2} M γ -CD volume (mL)	Final volume (mL)	$C_{\gamma\text{-CD}}$ (M)	RTP (520 nm) ^b
	1.00	40	0.00	10.0	0.00	
	1.00	40	0.50	10.0	2.00×10^{-3}	
	1.00	40	1.00	10.0	4.00×10^{-3}	
	1.00	40	1.50	10.0	6.00×10^{-3}	
	1.00	40	2.00	10.0	8.00×10^{-3}	
	1.00	40	2.50	10.0	1.00×10^{-2}	
	1.00	40	3.00	10.0	1.20×10^{-2}	
	1.00	40	3.50	10.0	1.40×10^{-2}	

^aThis determination was carried out using $\lambda_{\text{ex}} = 286$ nm. ^bThese columns are to be filled with the results obtained by the students.

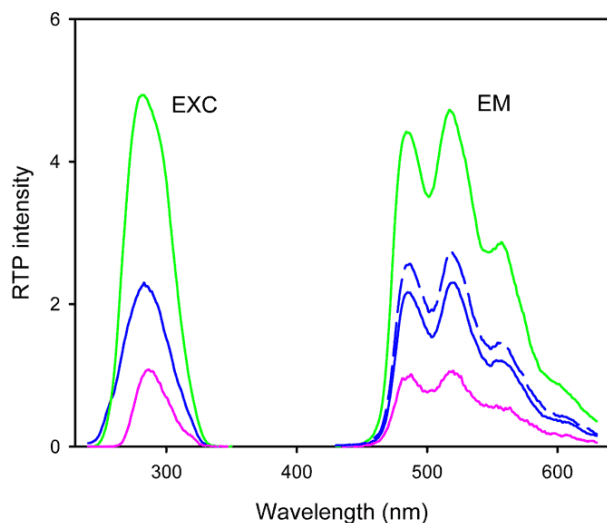


Figure 5. RTP spectra of acenaphthene ($C_{\text{AN}} = 1 \times 10^{-5}$ M) in the presence of (—) 8×10^{-3} M β -CD, 0.4% (v/v) 2,3-DBP, 0.04 M Na_2SO_3 ; (—) 0.014 M γ -CD, 0.4% (v/v) 2,3-DBP; (—) 2 M KI, 0.04 M Na_2SO_3 . The dashed line corresponds to the γ -CD system but in the presence of 0.04 M Na_2SO_3 .

iodide. In these cases, the following solutions were prepared: (1) 1×10^{-5} M AN, 0.014 M γ -CD, and 0.4% (v/v) DBP and (2) 1×10^{-5} M AN, 2 M KI, and 0.04 M sodium sulfite. It should be noted that in the β -CD system each sample must be previously irradiated with the spectrofluorimeter xenon lamp (approximately 15 min) at the excitation wavelength until the phosphorescence signal achieves its highest intensity. This fact has been observed in several phosphorescence systems and is rationalized in terms of photochemical catalysis of the deoxygenation reaction [14]. It should also be noted that deoxygenation with sodium sulfite is not necessary in the case of the γ -CD complex.

Lifetime Measurements. The above solutions prepared for the acquisition of the RTP spectra were also used to obtain decay curves and to measure the lifetimes. The instrumental conditions are shown in Table 2.

Ternary Association Constant. As an example of the procedure for determination of the association constant, the AN- γ -CD-2,3-dibromopropanol system was selected. Both AN and 2,3-dibromopropanol concentrations were held constant at 1.00×10^{-5} M and 0.4% (v/v), respectively, and the γ -CD concentration was varied from 0 to 1.4×10^{-2} M. Aliquots of a 1.0×10^{-4} M aqueous solution of AN and the

bromoalcohol were transferred to 10.00-mL flasks, the corresponding amounts of 0.04 M γ -CD solution were added, and the flasks were filled to the mark with double-distilled water. Table 3 shows the experimental solutions employed in the present example.

Results and Discussion

RTP Spectra. The excitation and emission phosphorescence spectra show the wavelengths where the maximum signals are obtained. Figure 5 illustrates the RTP spectra for AN from the different solutions. As can be seen in the figure, the signals produced in the β -CD system are the strongest. The system formed by γ -CD produces weaker signals, even in the presence of sodium sulfite as deoxygenator (see the dashed line spectrum in Figure 5). From Figure 5, it can also be concluded that AN phosphoresces more intensely in organized media than in media where it is not protected. It is apparent that the RTP emission is favored by the inclusion complex formation, because the nonradiative decay processes of the analyte are attenuated in the protected microenvironment of the CD.

Phosphorescence Lifetimes of AN. Figure 6 shows the phosphorescence decay of AN under the different conditions evaluated. Although in all cases a single exponential decay was observed, the decay rates corresponding to the protected and the unprotected media are very different. Correspondingly, the phosphorescence lifetimes for the AN in iodide solution and in the β - and γ -CD complexes have been determined to be 0.14, 30, and 40 ms, respectively. This fact is again justified by the efficient protection of the triplet state of the AN included in the CD cavity.

Conditional Equilibrium Constant Determination. Whereas in the absence of γ -CD no RTP emission was detected from the AN-DBP system, the phosphorescence intensity increased with increasing γ -CD concentrations. This fact confirms that the RTP emission is the result of a ternary complex formed between AN, γ -CD, and the bromoalcohol and that γ -CD is protecting the phosphorescing triplet state of AN from external quenchers and nonradiative pathways. The changes in the RTP emission of AN- γ -CD-DBP solutions are used to determine the conditional equilibrium constant for the reaction



In the presence of a large excess of alcohol, as in this case, the conditional equilibrium constant for the reaction is given by

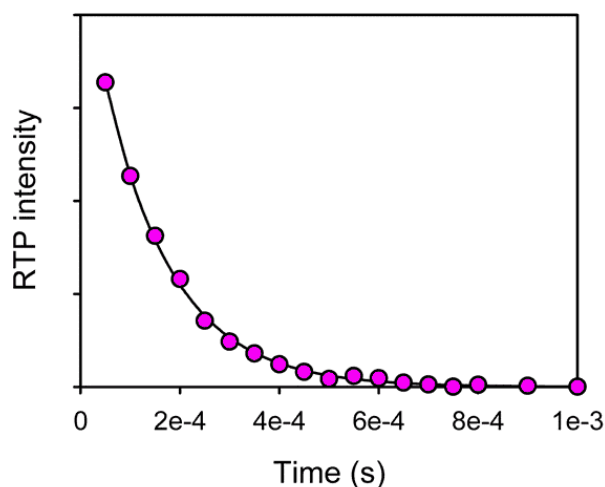
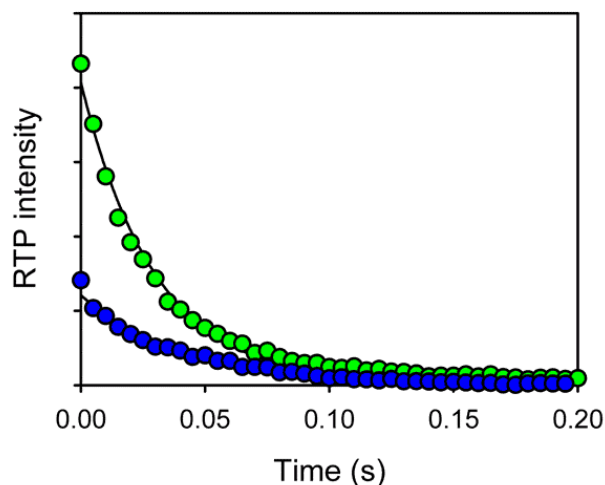


Figure 6. Decay curves corresponding to (●) β -CD system, (●) γ -CD system, and (●) iodide system. The solid line is the exponential fit of the data. The plots were split for clarity.

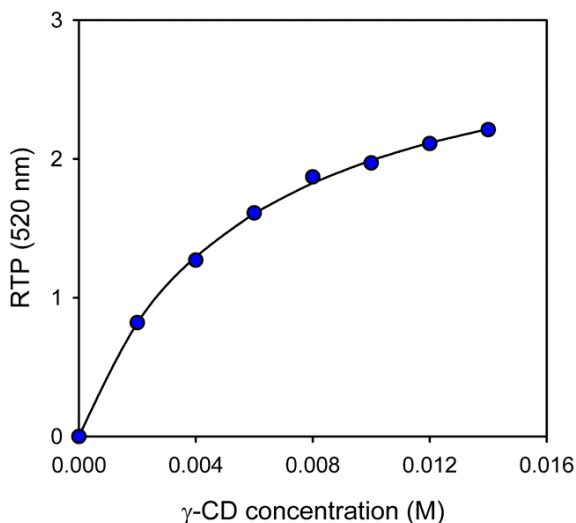


Figure 7. Influence of the γ -CD concentration on the RTP emission at 520 nm (exciting at 286 nm) of acenaphthene in the presence of 2,3-dibromopropanol. The solid line is the nonlinear fit of the data, assuming a 1:1 stoichiometry between the γ -CD and AN.

$$K' = \frac{[\text{AN-CD-DBP}_n]}{[\text{CD}][\text{AN}]} \quad (3)$$

Assuming that the concentration of the binary complex is negligible in all the experimental points and that the concentration of free CD is approximately equal to its analytical concentration (compare the total CD concentration used with that involved in the complex formation, which is limited by the low concentration of acenaphthene), the mass balance for AN can be written as

$$C_{\text{AN}} = [\text{AN}] + [\text{AN-CD-DBP}_n] \quad (4)$$

$$C_{\text{AN}} = \frac{[\text{AN-CD-DBP}_n](1 + K'C_{\text{CD}})}{K'C_{\text{CD}}} \quad (5)$$

Further, consider that the RTP signal is proportional to the ternary complex concentration, that is,

$$\text{RTP} = k[\text{AN-CD-DBP}_n] \quad (6)$$

and that at high γ -CD concentration the complex concentration is equal to the analytical concentration of AN, that is,

$$\text{RTP}_\infty = kC_{\text{AN}} \quad (7)$$

where k is a proportionality constant.

Substituting into eq 5,

$$\frac{\text{RTP}_\infty}{k} = \frac{\text{RTP}(1 + K'C_{\text{CD}})}{K'C_{\text{CD}}} \quad (8)$$

Rearranging eq 8, the following equation is obtained:

$$\text{RTP} = \frac{\text{RTP}_\infty K'C_{\text{CD}}}{1 + K'C_{\text{CD}}} \quad (9)$$

Finally, from the RTP emission of AN at 520 nm as a function of the γ -CD concentration (Figure 7) and using eq 9, K' can be easily calculated by a nonlinear regression method [21]. The equilibrium constant value obtained for the studied system was $K' = 179 \pm 7$.

Conclusion

The experiments discussed in this paper are examples of actual luminescence approaches to the acquisition of phosphorescence emission signals at room temperature in solution. We feel they are suitable for advanced undergraduate students, and they help them to appreciate new spectroscopic practices.

From these initial experiments, the work can be continued in various ways. For example, if analytical techniques are to be evaluated, students can perform the determination of AN by using phosphorescence signals in both artificial and real samples. Finally, this study should be completed using a rigorous statistical treatment.

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