

## Cyclodextrin-Enhanced Room-Temperature Phosphorescence

### Abstract

Cyclodextrin-enhanced room-temperature phosphorescence (CD-RTP) is an analytical technique that offers well-resolved spectra and subpicogram detection limits. When polynuclear aromatic hydrocarbons (PAHs) are included in cyclodextrin molecules in the presence of a heavy-atom moiety such as 1,2-dibromoethane, they exhibit intense, well-defined phosphorescence emissions, often with distinct vibrational structure. Moreover, the cyclodextrin moiety inhibits quenching of PAH phosphorescence by  $O_2$  present in the bulk solution.

### Introduction

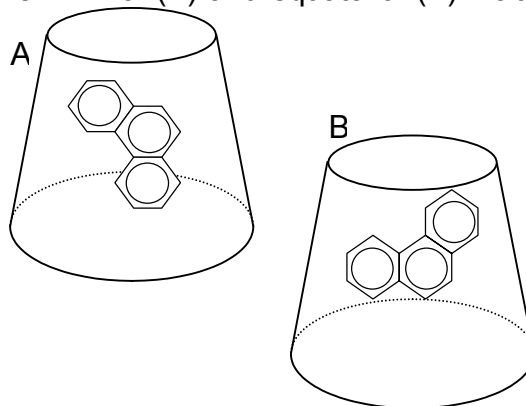
Cyclodextrins (CDs) are made up of glucose monomers coupled to form a rigid, conical structure with an interior hydrophobic cavity. The most common CDs, denoted by  $\alpha$ ,  $\beta$ , and  $\gamma$ , are composed, respectively, of six, seven, and eight monomers. Initially, each CD contains one or more water molecules, produced during the monomer coupling. Because these included water molecules are easily displaced by hydrophobic species that will fit into the CD interior, cyclodextrins have a unique ability to form stable inclusion complexes with a variety of molecules.

This inclusion capability has led to an important use of cyclodextrins in luminescence spectroscopy. Cyclodextrins have been found to enhance fluorescent and phosphorescent emissions from molecules present in the CD cavity.<sup>1,2,3,4,5</sup> Of particular interest is the phenomenon of cyclodextrin-enhanced room-temperature phosphorescence (CD-RTP), which suggests a highly-selective analytical technique based on the molecular geometry of the lumiphor.

Chromophores such as polynuclear aromatic hydrocarbons (PAHs) exhibit virtually no phosphorescence in conventional fluid media. Scypinski and

Cline Love report, however, that intense room-temperature phosphorescence occurs when these well-known carcinogens are present, with an external heavy atom, in the cavity of cyclodextrin.<sup>4</sup> Figure 1 depicts the inclusion of a phenanthrene molecule within the cavity of a  $\beta$ -CD. Scypinski and Cline Love also have observed CD-RTP in nitrogen heterocycles and bridged biphenyls.<sup>5</sup>

**Figure 1.** Axial (A) and equatorial (B) inclusion



of phenanthrene within the  $\beta$ -cyclodextrin moiety.

A description of the experiments with PAHs will serve to illustrate the technique of CD-RTP, and provide a basis for assessing its advantages.

### Experimental Method

Each PAH analyte was dissolved in either methanol or acetone, after which the solvent was gently evaporated. Addition of a 0.1–0.5-mL aliquot of 1,2-dibromoethane (DBE) was followed by dilution with 0.01-M aqueous CD solution. Upon vigorous shaking, the complex was formed. The cloudy solution obtained was then deoxygenated for fifteen minutes with nitrogen gas prepurified in an Alltech Oxy-Trap.

All spectra were taken on a SPEX® FLUOROLOG® spectrophotometer with double excitation and emission monochromators. A 450-W xenon CW lamp was used as the excitation source, and a cooled R928 photomultiplier (PMT) tube as the detector. Slits were set to 14.4 nm (excitation) and 3.6 nm (emission). The scan rate was 1 nm s<sup>-1</sup>.

<sup>1</sup> Hoshino, M.; Imamura, M.; Ikehara, K.; Hama, Y. *J. Phys. Chem.* **1981**, *85*, 1820.

<sup>2</sup> Turro, N.J.; Bolt, J.D.; Duroda, Y.; Tabushi, I. *Photochem. Photobiol.* **1982**, *35*, 69.

<sup>3</sup> Turro, N.J.; Cox., G.S.; Li, X. *Photochem. Photobiol.* **1983**, *37*, 149.

<sup>4</sup> Scypinski, S.; Cline Love, L.J. *Anal. Chem.* **1984**, *56*, 322.

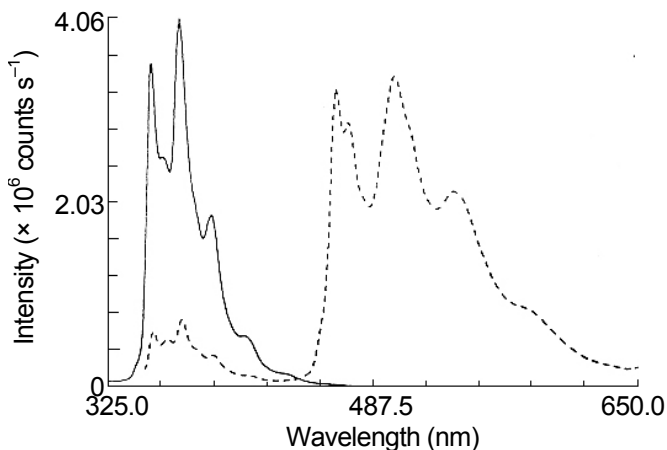
<sup>5</sup> Scypinski, S.; Cline Love, L.J. *Anal. Chem.* **1984**, *56*, 331.

All emission spectra were corrected for fluctuations in the intensity of the lamp and PMT responses.

## Results and Discussion

The investigation showed the CD-RTP technique to be extremely sensitive, producing intense, well-structured phosphorescence signals at nanomolar concentrations. Detection limits of two typical phosphors, phenanthrene and acenaphthene, were estimated at  $5 \times 10^{-13} M$  and  $1 \times 10^{-11} M$ , respectively. Although the excited triplet state is known to be susceptible to oxygen quenchers, inclusion of the lumiphor inside the CD cavity was observed to offer some protection against quenching by oxygen molecules in the bulk solution. In these experiments, 10% to 40% of the RTP remained after aeration of the sample—a signal still strong enough for analytical purposes.

The success of CD-RTP with PAHs depends on the formation of a proposed three-component complex of CD, lumiphor, and external heavy atom. The internal cavity of the CD must be large enough to accommodate both the lumiphor, which must fit at least partially inside, and the heavy atom.

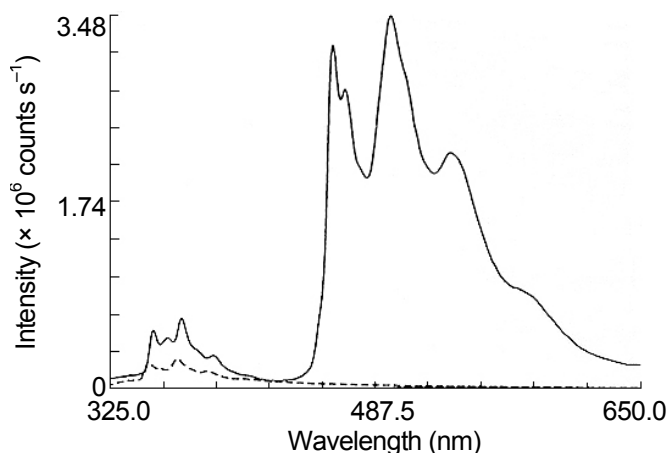


**Figure 2.** Corrected emission spectra of  $5 \times 10^{-5}$ -M phenanthrene in 0.01-M  $\beta$ -CD alone (solid line) and with 0.58-M DBE (dashed line). Excitation wavelength = 300 nm.

Figure 2 shows the luminescence spectra for phenanthrene included in  $\beta$ -CD, with and without the addition of the “heavy-atom” DBE. Because the heavy-atom moiety enhances spin-orbit coupling and the rate of inter-system crossings, addition of

DBE serves to quench much of the fluorescence while intensifying the phosphorescence.

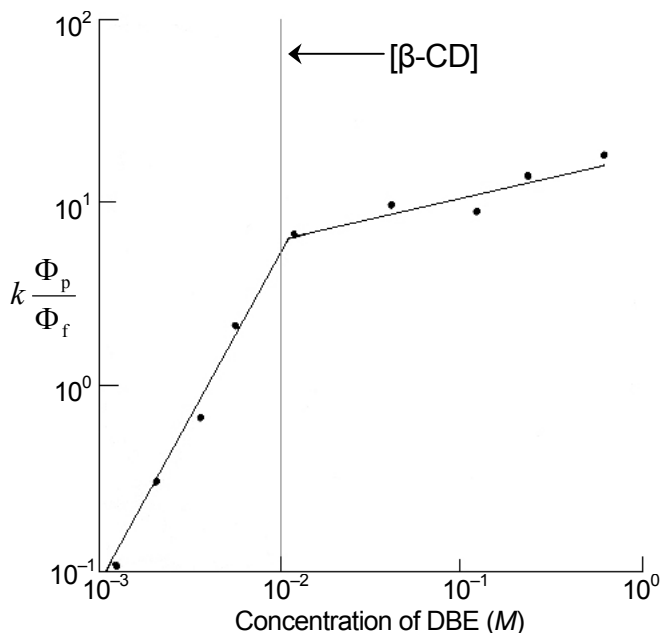
The physical dimensions of the CD cavities, relative to the geometry of the individual PAH, determined the most effective CD form for each analyte. For example, although the phenanthrene molecule will fit inside the cavity of the  $\alpha$ -CD, which has an inner diameter of 0.6 nm, there is apparently not enough room remaining to accommodate the DBE molecule. Both phenanthrene and DBE molecules, however, can be included in the larger  $\beta$ -CD cavity, which has an inner diameter of 0.78 nm. Figure 3 compares the luminescence spectra for the same solution of phenanthrene and DBE in  $\alpha$ - and  $\beta$ -CD. Anthracene, which is larger than phenanthrene, showed only weak phosphorescence in  $\beta$ -CD, but  $\gamma$ -CD, with an inner diameter of 1.0 nm, induced stronger emissions.



**Figure 3.** Corrected emission spectra of  $5 \times 10^{-5}$ -M phenanthrene in 0.01-M  $\beta$ -CD (solid line) and in 0.01-M  $\alpha$ -CD (dashed line). Excitation wavelength = 300 nm; [DBE] = 0.58 M.

The geometrical limitations imposed by CD-RTP may be exploited as a selective means of analysis based on molecular size and shape. For example, it is possible to discriminate between naphthalene, which can be induced to phosphoresce in  $\beta$ -CD, and its bulkier derivative 1-phenylnaphthalene, which shows only fluorescence under the same conditions.

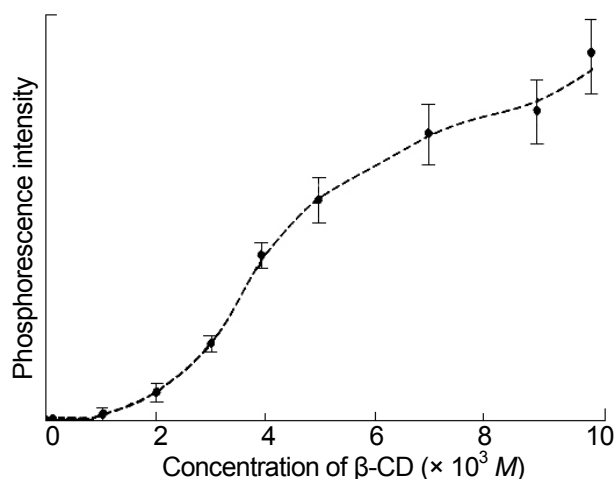
In these experiments, the phosphorescence intensity of the included PAH was found to depend



**Figure 4.** Dependence of the relative phosphorescence intensity of  $5 \times 10^{-5}$ -M phenanthrene on the concentration of DBE in 0.01-M  $\beta$ -CD.  $k(\Phi_p/\Phi_f)$  is the ratio of the integrated intensities of the corrected phosphorescence to corrected fluorescence spectral profiles. Excitation wavelength = 330 nm.

on the concentration of the heavy-atom moiety and on the concentration of CD. Figure 4 illustrates the relationship of the phosphorescence intensity of phenanthrene to the concentration of DBE. As long as the concentration of DBE is low with respect to that of CD, DBE molecules added into the solution are incorporated in the trimolecular inclusion complex, causing a sharp increase in phosphorescence intensity. When the DBE concentration reaches that of the CD, so that complexation is virtually complete, further addition of DBE enhances the phosphorescence only slightly.

Figure 5 demonstrates the dependence of phosphorescence intensity on the concentration of CD. The S-shaped curve traces the phosphorescence intensity of the phenanthrene-DBE solution as  $\beta$ -cyclodextrin is added. The initial steep slope has



**Figure 5.** Dependence of phosphorescence intensity (number of photons) of  $5 \times 10^{-5}$ -M phenanthrene on the concentration of  $\beta$ -CD with 0.58-M DBE present. Excitation wavelength = 300 nm, (error bars are  $\pm 10\%$  RSD); emission wavelength = 501 nm.

been attributed to the sharply-increasing probability that a phenanthrene molecule exiting the cavity of a CD-DBE molecule will find another CD-DBE molecule in close proximity.<sup>4</sup>

## Conclusion

Cyclodextrin-enhanced room-temperature phosphorescence shows promise as a sensitive analytical technique. Sample preparation is simple, data-acquisition and processing are easily automated with a FLUOROLOG® spectrometer, and the result is an intense, well-structured RTP signal. CD-RTP offers the advantages of increased selectivity based on molecular geometry, and partial immunity to quenching by dissolved  $O_2$  molecules.

## Acknowledgement

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