



Measurements of Room-Temperature Phosphorescence Spectra of Polycyclic Aromatic Compounds Using Pullulan Films Containing β -Cyclodextrin and 2-Bromoethanol

MASAKI TACHIBANA*, KAZUE TANI, HITOSHI KOIZUMI and NOBUTOSHI KIBA

Department of Applied Chemistry and Biotechnology, Faculty of Engineering, Yamanashi University, 4-3-11 Takeda, Kofu, Yamanashi 400-8511, Japan

(Received: 1 March 1999; in final form: 8 July 1999)

Abstract. A clear film was easily prepared by air-drying an aqueous solution of pullulan (6% w/w) containing β -cyclodextrin (CD, 1% w/w) and 2-bromoethanol (1% v/w). The resulting pullulan film was used as a substrate for simple measurements of room-temperature phosphorescence (RTP) spectra of polycyclic aromatic compounds (PACs). Only a drop (ca. 10 μ L) of a 100 μ g mL⁻¹ sample solution in 95% ethanol was spotted onto the surface of the disk film (7–8 mm diameter) and the solvent was allowed to evaporate at room temperature. The sample-spotted film was pasted on a glass plate (75 \times 20 \times 1 mm) with small amounts of a starch glue. The plate was mounted into a solid sample holder, or alternatively inserted diagonally into a 1-cm cell holder. Without a dry gas flush during the measurements, RTP spectra based on the CD inclusion complexes of PACs were obtained from six typical two- and three-ring compounds, including naphthalene, acenaphthene, fluorene, phenanthrene, carbazole, and dibenzofuran. Only anthracene did not produce a discernable phosphorescence signal by the present technique. This technique was directly applied to the spectral identification of acenaphthene in commercially available kerosene.

Key words: room-temperature phosphorescence spectra, pullulan films, β -cyclodextrin, 2-bromoethanol, polycyclic aromatic compounds

1. Introduction

Room-temperature phosphorescence (RTP) spectrometry has been recognized as a sensitive, selective, and inexpensive technique for the characterization and analysis of trace amounts of numerous organic compounds and has therefore been reviewed in detail within the last few decades [1–4]. It is generally accepted in these investigations that a phosphor has to be held tightly in a rigid microenvironment for strong RTP to occur. Therefore, applications of the RTP technique to polycyclic aromatic compounds (PACs) have been developed into several types based on the differences in the rigid environments, including micelle-stabilized [5, 6], microemulsion [7,

* Author for correspondence.

8], cyclodextrin-induced [9, 10], and solid-surface and -matrix RTP spectrometry [11, 12]. Filter papers have appeared to be the most commonly available substrates for the solid-matrix RTP measurements of various species at the present stage. However, one problem of paper substrate RTP is that the opaque material not only gives appreciable background signals, but also sophisticated sample handling is needed, with specially designed holders or supports.

This paper describes the development of a new solid-matrix technique for convenient RTP measurements of PACs, using a three-component film made up of pullulan, β -cyclodextrin (CD), and 2-bromoethanol. Pullulan is a viscous polysaccharide produced extracellularly by the fungus *Aureobasidium pullulans* (also known as *Pullularia Pullulans*) from glucose or other saccharides [13, 14]. Pullulan consists of maltotriose units, i.e. units of three α -1,4-linked glucose molecules, which are polymerised in a linear fashion via α -1,6-linkages. Manufacturers are producing the polymer in the molecular weight range from 10 000 to 400 000, and therefore pullulan with an average molecular weight of 50 000–200 000 is readily available in a pure form. Purified pullulan is a colorless, water-soluble, tasteless and odorless powder, and applications have been found in the production of nontoxic membranes, films, or fibers in the food industry [15].

Clear pullulan films containing CD and 2-bromoethanol were easily prepared by air-drying an aqueous solution of their mixture. The CD component in the film is well known for its ability to form inclusion complexes with a variety of PACs on the basis of spatial fitting. This property of CD has been effectively employed to induce the RTP signals of PACs with the aid of a heavy atom, such as that in 2-bromoethanol [16, 17].

In the present research the use of the clear pullulan film was restricted within qualitative RTP approaches because of poor reproducibility. The poor reproducibility may be attributable to a departure from optimum conditions or the inadequacy of the proposed procedure for individual PACs, including film preparation, solvent choice, and RTP measurement. However, it is possible to a certain extent to achieve higher precision by modifying the procedure.

The present technique possesses outstanding characteristics in terms of simplicity and convenience for RTP investigations of PACs. The pullulan films do not require tedious pre-treatments before use, nor a dry gas flush during measurements, both of which are ordinarily required for filter papers. Additionally, it is possible to measure the RTP radiation transmitted through the clear material by using a normally equipped 1-cm cell holder without special attachments. This novel means of obtaining RTP signals of PACs will lead to increased diversity and flexibility in RTP research.

2. Experimental

2.1. APPARATUS

All RTP measurements were performed on a Hitachi F-4500 fluorescence spectrophotometer with a rotary chopper for the acquisition of phosphorescence data and using a solid sample-holder attachment. The spectrophotometer (without the use of such special accessories) was also used in some cases to obtain RTP and room-temperature luminescence (RTL) spectra. Both excitation and emission slits for the RTP and RTL measurements were set at 10 and 5 nm, respectively. A 150-W xenon arc lamp was used as the excitation source. For one spectral measurement, three runs were carried out with a scan rate of 240 nm min^{-1} and the data were processed by averaging through a Hewlett-Packard Vectra 286/12 computer interfaced with the spectrophotometer.

2.2. REAGENTS

Pullulan and β -CD of guaranteed-reagent grade were purchased from Tokyo Kasei Kogyo Ltd. (Tokyo, Japan) and used as received without further purification. Distilled water and ethanol (95%) were of analytical-reagent grade and 2-bromoethanol was of guaranteed-reagent grade (Kanto Chemical Inc., Tokyo, Japan); these solvents were used as received. Commercially available three-ring PACs were finally purified by zone melting after the removal of main impurities with appropriate pre-treatments, including the oxidative treatment for fluorene in acetic acid solutions with chromium (VI) oxide, the Diels-Alder reaction of anthracene with maleic anhydride, and the extraction of carbazole from benzene solutions with 85% (w/w) sulfuric acid [18]. Naphthalene was zone-refined without such chemical pre-treatments. Working standard solutions of PACs in 95% ethanol were directly prepared at a concentration of ca. $100 \mu\text{g mL}^{-1}$ by dissolving about 2 mg of each PAC sample in a 20-mL volumetric flask and diluting to the mark. These standard solutions were kept at ambient temperature in the dark throughout the present research.

2.3. FILM PREPARATION

All disposable films were easily prepared according to the following manner: 600 mg of pullulan, 100 mg of CD, and 9.3 g of distilled water were mixed and stirred in a 50-mL Erlenmeyer flask with a glass stopper. After the powdered compounds were dissolved completely, 0.1 mL of 2-bromoethanol was added to the solution and stirred thoroughly. The final concentrations of the three components in the aqueous solution were 6% (w/w) for pullulan, 1% (w/w) for CD, and 1% (v/w) for 2-bromoethanol. Portions (0.1 mL) of the viscous solution were carefully poured onto the flat surface of an ordinarily available poly(vinyl chloride) (PVC) lining sheet (ca. 1 mm in thickness) with a measuring pipette to form small, separate droplets.

The water in the solution delivered onto the PVC sheet was then evaporated at atmospheric pressure and ambient temperature to produce clear films. All of the films prepared were further kept drying in air on the PVC sheet overnight, and finally covered with another PVC sheet in order to sandwich them firmly between the sheets. These sandwich films were stored in a dark drawer until use. The resulting film was a clear disk (7–8 mm diameter) having a slightly uneven surface and a smooth reverse side, due to the simple air-drying of a droplet on a flat PVC sheet.

2.4. MEASUREMENT PROCEDURE

With a Pasteur pipette, one drop only (ca. 10 μL) of the standard PAC solution (ca. 100 $\mu\text{g mL}^{-1}$) was gently added onto an uneven surface of the film put on a filter paper, without spilling out over the surface. The solvent delivered onto the film surface was then evaporated for at least 10 min at room temperature. With a pair of tweezers, the resulting solvent-free film was handled and fixed at the excitation location on a glass plate (75 \times 20 \times 1 mm) where small amounts of an ordinary starch glue had been previously spread. After standing for a while in the dark, the film-pasted plate was normally fitted into a solid sample holder, or, with more convenience, diagonally in a common 1-cm quartz cell holder of the spectrofluorimeter. The RTP and/or RTL spectra of the PAC sample adsorbed on the top of the clear film were measured without dry gas flush, using appropriate excitation and emission wavelengths for each PAC.

3. Results and Discussion

3.1. RTP SPECTRA

Seven typical two- and three-ring aromatic compounds were deposited on the pullulan films containing CD and 2-bromoethanol according to the procedure described above. The individual film treated with a standard PAC solution was pasted onto a glass plate, and then it was allowed to stand for a few hours or days in order to acquire a reasonable RTP signal. In the present work, the spectral measurements of PACs spotted on the films were carried out about five hours later, as a matter of convenience, because of the apparent difference in optimum standing time for the PACs. By mounting the plate in a solid sample holder, the phosphorescence diffusely reflected from the film surface was measured at room temperature (22–24 $^{\circ}\text{C}$), without a dry gas flush. Figure 1 shows the RTP spectra of all of compounds tested, except anthracene, including phenanthrene, acenaphthene, naphthalene, fluorene, carbazole and dibenzofuran, and of the corresponding blanks. Since anthracene only did not produce a discernable phosphorescence intensity using the present procedure, it was difficult to obtain its RTP spectrum. The strong RTP signals of the other six PACs imply the formation of a ternary inclusion complex of CD, the PAC, and the heavy atom in the solid material. Very weak RTP or no RTP has been reported for anthracene in α -CD and β -CD inclusion

systems with heavy atoms [9, 19], while an increased phosphorescence signal has been observed in γ -CD in the presence of 1,2-dibromoethane [9]. Therefore, it may be possible to measure the RTP of anthracene by using γ -CD as an alternative to β -CD for the film preparation. These results demonstrate that our novel pullulan/CD/2-bromoethanol films are useful as a solid-matrix substrate for RTP studies of PACs.

3.2. VARIATION OF RTP INTENSITY

In order to investigate the stability of PAC phosphorescence on the pullulan/CD/2-bromoethanol film, RTP intensities of phenanthrene (ca. 1 μg) were measured repeatedly with a suitable time interval under the same conditions. The results of the two runs are shown in Figure 2. The other PACs studied had almost similar phosphorescence behavior to phenanthrene. Figure 2 reports that the standing time after dropping of the sample solution is an important factor in affecting the RTP intensity of the phosphor deposited on the film. Also, it is found that the intensities do not go through a reproducible value during a few days, while no appreciable decrease in the RTP signals was observed, even after a week. The reason for such significant variation of the PAC phosphorescence is not clear at present. A number of factors may be involved in clarifying the fluctuation phenomenon of the RTP signals. There seem to be a number of parameters based on the RTP characteristics of PACs and/or the CD interactions with PACs, including drying of the film surface and bulk, evaporation and diffusion of the solvent, phosphor-substrate interactions, hydrogen bonding network in the substrate, CD and 2-bromoethanol dispersions in pullulan, escape of the volatile heavy atom from the matrix, and inclusion equilibrium between CD and PAC. Further studies on the stabilization of the RTP intensities of PACs dropped on the film are currently under way.

3.3. MEASUREMENTS OF TRANSMITTED LUMINESCENCE

The most striking characteristic of the pullulan/CD/2-bromoethanol film is the transparency of the solid material. It is consequently possible to acquire the RTP radiation transmitted through the clear film as well as the radiation diffusely reflected from the surface. The measurements of the transmitted luminescence radiation possess several intrinsic advantages compared with those of the reflected radiation. First, background emission intensities of the films should be reduced because most of the light scattering takes place on the solid surface. Such a background reduction is of great importance in trace analysis. An additional, experimental advantage is that a 1-cm quartz cell holder, which is the most usual sample holder in many different spectrofluorimeters, can be utilized for RTP and RTL measurements.

A phenanthrene-deposited film was pasted on a glass plate ($75 \times 20 \times 1$ mm) with small amounts of a starch glue. The plate was inserted vertically into a 1-cm cell holder at an angle of 45° (diagonal position) to the direction of incidence. All

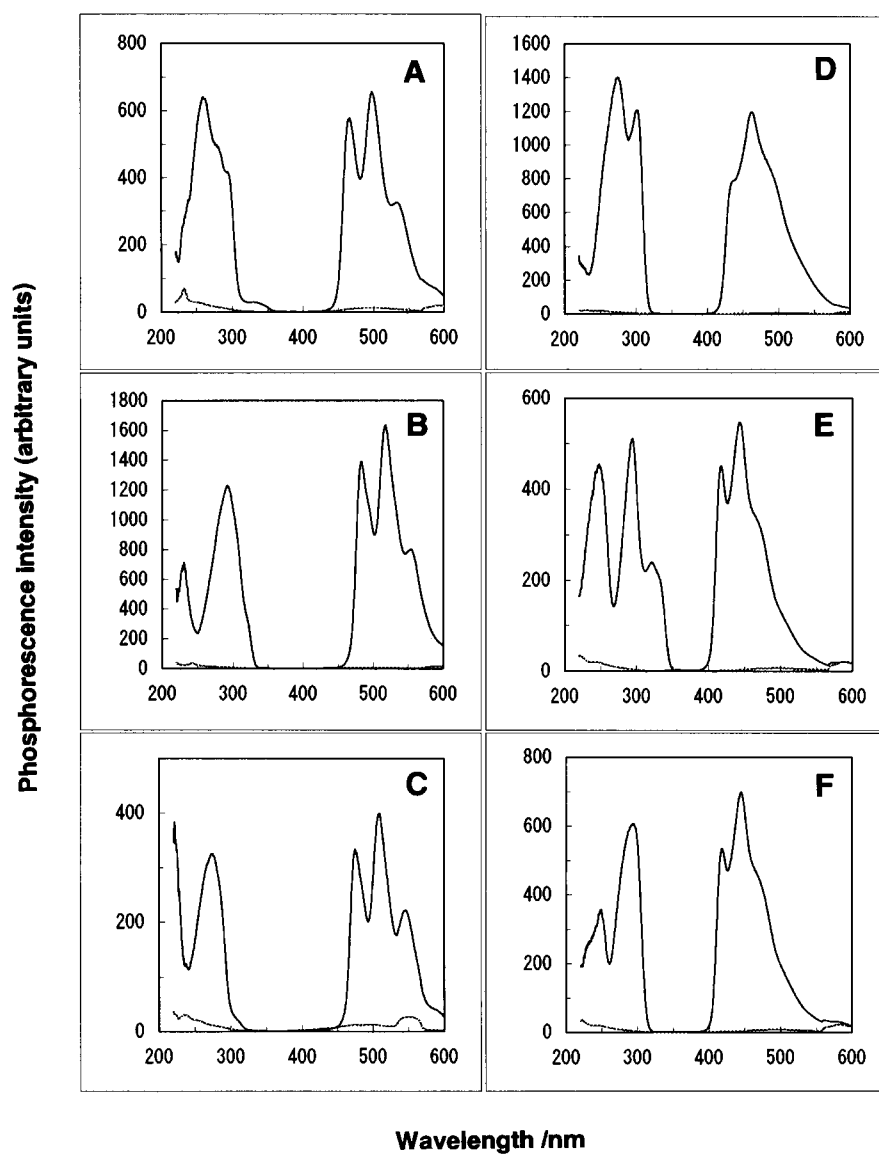


Figure 1. RTP excitation and emission spectra of: (A) Phenanthrene; (B) acenaphthene; (C) naphthalene; (D) fluorene; (E) carbazole; (F) dibenzofuran (ca. 1 μg) spotted on pul-lulan/CD/2-bromoethanol films and of their blanks. All of the spectra were obtained by measuring the radiation diffusely reflected from the film surface at 22–24 $^{\circ}\text{C}$, and are uncorrected.

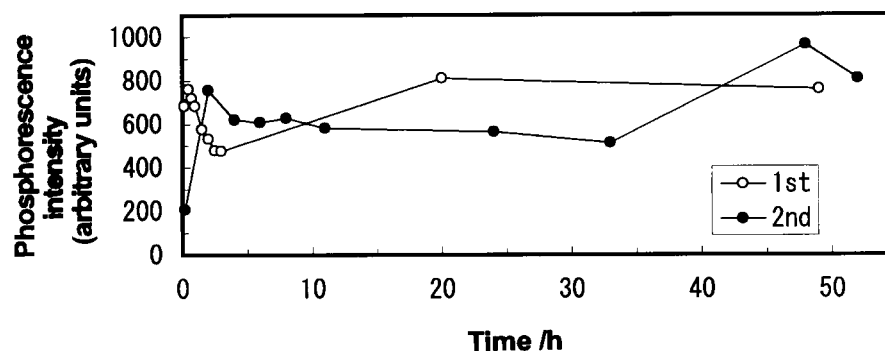


Figure 2. Variation of the RTP intensity of phenanthrene (ca. 1 μg) after dropping of the 95% ethanol solution on the film surface. The RTP intensities were measured with $\lambda_{\text{ex}} = 295 \text{ nm}$ and $\lambda_{\text{em}} = 498 \text{ nm}$ at 22–24 $^{\circ}\text{C}$. The run was repeated once more under the same conditions.

of the radiation transmitted through both the film and the plate was then measured using an excitation wavelength of 295 nm. The luminescence emission spectra of phenanthrene thus obtained are given as (a) in Figure 3 and further the film blank as (c). The two spectra indicate that the observed RTL of phenanthrene consists of residual fluorescence and induced phosphorescence, and it is not so much influenced by the film background emission. The spectrum (b) in Figure 3 is measured on the same film of the sample via additional processes, in which a drop of 2-bromoethanol was added onto the surface of the film pasted on the plate and the heavy atom was evaporated in air. As can be seen by comparing these spectra (a) and (b), the additional spotting of 2-bromoethanol resulted in a greater than about twofold increase in the RTP signal of phenanthrene. It can be concluded that the 2-bromoethanol post-dropping on the film is effective for RTP enhancement, presumably because of the higher probability of very close contact of the heavy atom with the phosphor in a CD cavity.

3.4. CHARACTERIZATION OF ACENAPHTHENE IN KEROSENE

The proposed RTP technique was directly applied to characterization of PACs in commercially available kerosene fuels. A drop of a 10% kerosene solution in 95% ethanol was transferred to the surface of the pullulan/CD/2-bromoethanol film. After drying in air for 0.5 hour, the sample-spotted film was pasted on a glass plate and the plate was diagonally fixed in a 1-cm cell holder. The phosphorescence emission transmitted through the clear film was then measured with an excitation wavelength of 295 nm. Twenty hours later, in addition, the measurement of this spotted film was repeated once again under the same conditions. These spectra obtained after standing for 0.5 and an additional 20 hours are given in Figure 4 as (a) and (b), respectively.

As can be seen from the two spectra, the kerosene-deposited film does not give a strong RTP signal, satisfactory for PAC characterization, although weak peaks at

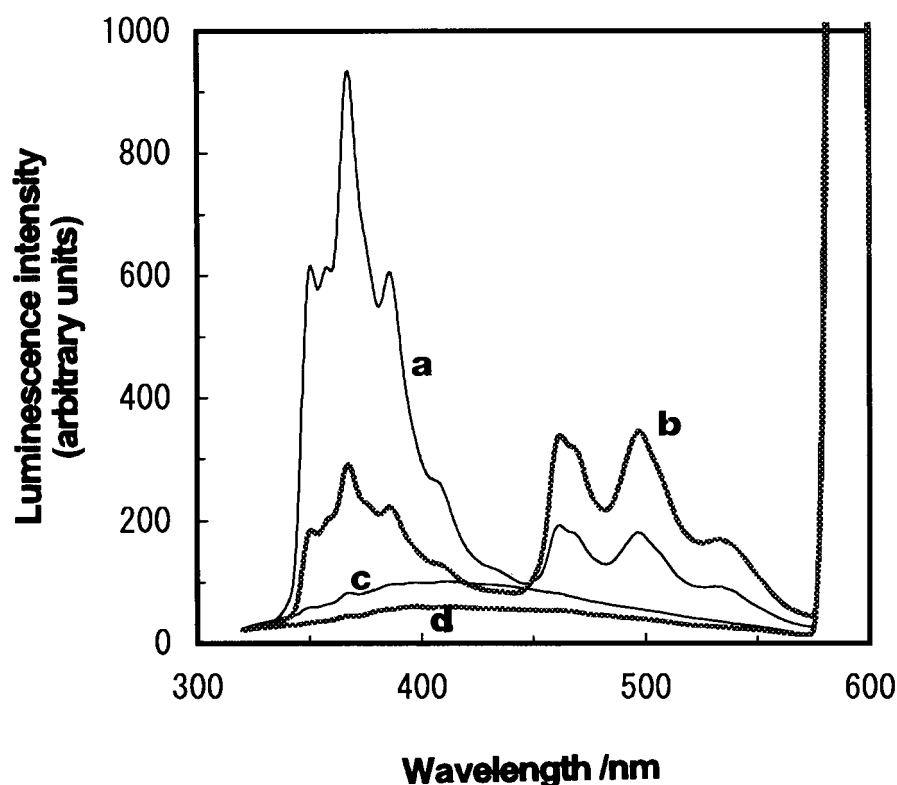


Figure 3. Comparison of transmitted RTL emission spectra ($\lambda_{\text{ex}} = 295$ nm, uncorrected temp. 22–24 °C) of phenanthrene (ca. 1 μg): (a) without and (b) with additional 2-bromoethanol dropping on the film, and of the blank (c) without and (d) with additional 2-bromoethanol.

483 and 517 nm are observable. In order to enhance the weak RTP signal from the sample film by increasing the interaction between the phosphor and heavy atom, the 2-bromoethanol post-dropping process was further performed on this film. The phosphorescence spectrum thus obtained is shown in Figure 4(c) and compared with that of an acenaphthene 10 $\mu\text{g mL}^{-1}$ solution (spectrum d). From analogy between these two RTP spectra the trace existence of acenaphthene in the commercial kerosene fuel can be inferred to some extent. Kerosene is a complicated mixture of petroleum hydrocarbons, chiefly of the alkane series having 10–16 carbon atoms per molecule. Though various analytical methods have been developed in order to assess the quality, the identification and/or determination of a given PAC contained as a trace constituent has been a formidable task in any procedure. However, trace amounts of acenaphthene in commercially available kerosene had been already determined by a selective solvent extraction/synchronous spectrofluorimetric technique [20]. The presence of acenaphthene observed in the present research is consistent with the result obtained from the selective analytical method.

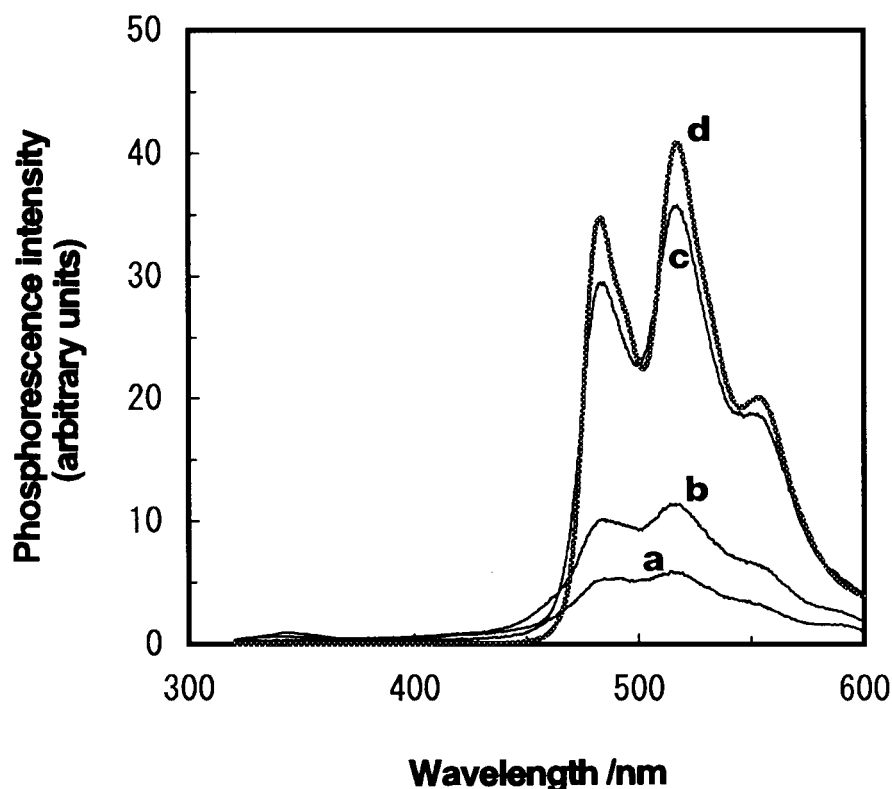


Figure 4. Transmitted RTP emission spectra ($\lambda_{\text{ex}} = 295$ nm, uncorrected temp. 22–24 °C) of kerosene (ca. 1 μL) after standing time for: (a) 0.5 hour; (b) additional 20 hours; (c) with further 2-bromoethanol post-treatment, and of (d) acenaphthene spotted on pullulan/CD/2-bromoethanol films.

4. Conclusion

The spectral data presented here demonstrate that the three-component film which consists of pullulan, β -CD, and 2-bromoethanol is useful as a solid-matrix substrate for RTP investigations of PACs. Film preparation is simple and several dozen films can be formed as disks during one preparation process. The disks thus obtained are colorless, transparent, inexpensive, and disposable. By spotting on the surface of the disk films, discernable RTP signals were obtained from all of the six typical two- and three-ring aromatic compounds studied (except anthracene). In addition to the diffusely-reflected RTP radiation from the film surface, it was also possible to measure the lower-background radiation transmitted through the clear film. The advantages of the present film over other solid-matrix substrates are that the experimental procedure is simple and convenient for measurements of the RTP spectra of PACs.

References

1. T. Vo-Dinh: *Room-Temperature Phosphorimetry for Chemical Analysis*, Wiley, New York (1984).
2. R. J. Hurtubise: *Phosphorimetry: Theory, Instrumentation, and Applications*, VCH, New York (1990).
3. M. Gunsheski, J. J. Santana, J. Stephenson, and J. D. Winefordner: *Appl. Spectrosc. Rev.* **27**, 143 (1992).
4. R. J. Hurtubise: *Anal. Chim. Acta* **351**, 1 (1997).
5. K. Kalyanasundaram, F. Grieser, and J. K. Thomas: *Chem. Phys. Lett.* **51**, 501 (1977).
6. W. Jin and C. Liu: *Microchem. J.* **48**, 94 (1993).
7. G. R. Ramos, I. M. Khasawneh, M. C. Garcia-Alvarez-Coque, and J. D. Winefordner: *Talanta* **35**, 41 (1988).
8. A. S. Carretero, C. C. Blanco, and A. F. Gutierrez: *Anal. Chim. Acta* **353**, 337 (1997).
9. S. Scypinski and L. J. C. Love: *Anal. Chem.* **56**, 322 (1984).
10. W. Jin, Y. Wei, A. Xu, and C. Liu: *Spectrochim. Acta* **50A**, 1769 (1994).
11. T. Vo-Dinh, E. L. Yen, and J. D. Winefordner: *Talanta* **24**, 146 (1977).
12. S. W. Tjioe and R. J. Hurtubise: *Talanta* **41**, 595 (1994).
13. P. Prave, U. Faust, W. Siggig, and D. A. Sukatsch: *Handbuch der Biotechnologie* (3rd edn.), p. 378, R. Oldenbourg Verlag, Munchen (1987).
14. A. LeDuy, L. Choplin, J. E. Zajic, and H. T. Luong: In *Encyclopedia of Polymer Science and Engineering* (Vol. 13), pp. 650–660, Wiley, New York (1988).
15. S. Yuen: *Proc. Biochem.* **9**, 7 (1974).
16. S. Hamai: *J. Am. Chem. Soc.* **111**, 3954 (1989).
17. A. Munoz de la Pena, I. Duran-Meras, F. Salinas, I. M. Warner, and T. T. Ndou: *Anal. Chim. Acta* **255**, 351 (1991).
18. M. Tachibana and M. Furusawa: *Analyst* **119**, 1081 (1994).
19. S. M. Ramasamy and R. J. Hurtubise: *Appl. Spectrosc.* **50**, 115 (1996).
20. M. Tachibana and M. Furusawa: *Analyst* **120**, 437 (1995.)