

An Introduction to Phosphorescence Spectroscopy

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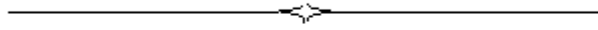
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Introduction

Phosphorescence has been observed from a wide variety of compounds and is differentiated from fluorescence by the long-lived emission of light after extinction of the excitation source. The first analytical uses of phosphorescence were published in 1957 by Kiers et al [1]. However, the technique has still not been widely accepted apart from a few selected areas such as pharmaceutical analysis and forensic science. The reluctance to use phosphorescence probably arises from the practical aspect of measuring the signal since cryogenic temperatures, using liquid nitrogen at 77 K, are normally required. Recent developments in room temperature phosphorescence (RTP) have given rise to practical and fundamental advances which should help stimulate interest in phosphorimetry.

The sensitivity of phosphorescence is comparable to that of fluorescence and complements the latter technique by offering a wider range of molecules for study. As well as offering selectivity through the use of excitation and emission wavelengths, phosphorescence adds another dimension - that of time. Background interference from fluorescence and Rayleigh and Raman scattering can be rejected on a time basis. Fluorescence to phosphorescence ratios can be used to aid the identification of particular compounds and the purity of organic compounds can be determined by measuring their decay times.

The introduction of a microprocessor based phosphorimeter using a pulsed xenon source will provide the analyst with much greater freedom and ease of operation which should stimulate greater interest in the technique.

Theory

The ground state of most organic compounds is the singlet state S_0 and the absorption of light promotes the electrons within the molecule to any one of several excited states S_1 , S_2 , S_n , depending upon the wavelength (frequency) of the radiation. This absorption process takes about 10^{-15} sec to occur and whilst in this excited state the molecule will rapidly relax ($10^{-12} - 10^{-14}$ sec) by a non-radiative route (internal conversion) to the lowest excited singlet state S_1 . From this S_1 level, three possible routes can return the molecule to the ground state.

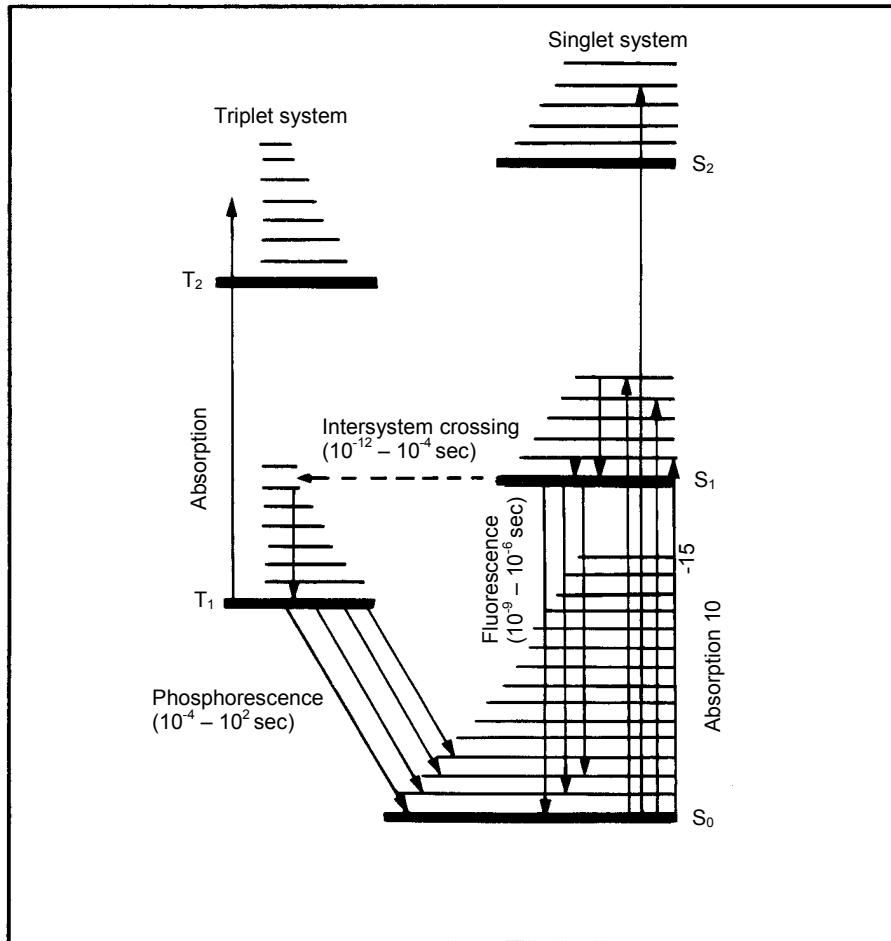


Figure 1 Simplified energy level diagram of a polyatomic molecule

A non-radiative internal conversion (10^{-5} to 10^{-7} sec) can take place with the excess energy released as heat. A radiative transition can occur (10^{-6} to 10^{-9} sec) to the various vibrational states that form the ground state S_0 and this is called fluorescence. [2]

In molecules where the singlet S_1 and triplet T_1 energy levels are closely spaced the molecule can drop into the lower energy T_1 state through a process known as intersystem crossing (10^{-8} sec). The molecule can then return to the ground state by emission of radiation and this T_1 to S_0 transition is called phosphorescence. The latter has a long life-time which can vary from 10^{-6} to 10^2 sec depending upon the structure of the molecule. Phosphorescence emission spectra occur at longer wavelengths than fluorescence emission spectra because of the slight loss in energy which occurs in going from the singlet to triplet state.

Because of the long life-times, the molecule has a very high probability of losing its excess energy by radiativeless routes such as internal conversion, bimolecular collision, and photodecompositions. As a result, phosphorescence is not routinely observed in solutions at room temperature. Quenching of the triplet state by oxygen is also effective in preventing phosphorescence, and thorough degassing of the solution is required before measurement.

Several methods have been used to enable the observation of phosphorescence, in other words, to restrict collisional deactivation. One of the most common techniques is to supercool solutions to a rigid glass state usually at the temperature of liquid nitrogen (77 K). At these low temperatures, molecular collisions are greatly reduced and strong phosphorescence signals are observed. Fluorescence can also be observed at low temperatures with a sharpening of the emission peaks through the Shpol'skii effect.

Phosphorescence can also be observed by inserting the analyte into a rigid polymer matrix, although the area of applications is limited. The phosphorescence of coronene embedded in a polymethylmethacrylate block can be observed but only after oxygen, which may have diffused into the block, has been removed by heating the block at 100 °C for 12 hours (Figure 2).

Recent developments have shown that, under certain circumstances, the phosphorescence of polyatomic aromatic compounds adsorbed on a variety of supports can be observed at room temperature. In 1972 Schulman and Walling [3] published two papers indicating that phosphorescence of sufficient strength for analytical purposes could be observed from polar organic molecules adsorbed onto filter paper, silica and other chromatographic supports. Since their publication, there have been a number of other fundamental and practical advances which have increased the interest in room temperature phosphorescence (RTP). Amongst these advances, the most important feature is the addition of heavy atoms which improves the sensitivity of the technique.

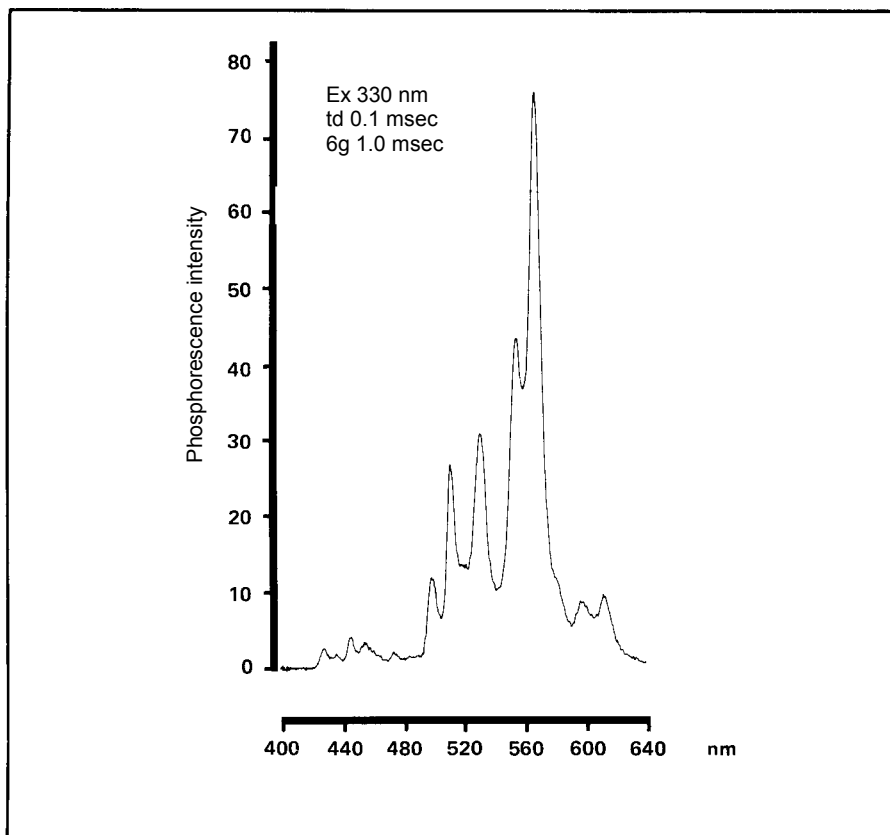


Figure 2 Phosphorescence emission spectrum of coronene in PMMA

The added dimension of time imparts a number of advantages for the analyst. Stray light from the excitation monochromator is rejected in the phosphorometric mode, as is second order scattering. In addition Raman bands no longer interfere with the analysis since the Raman phenomenon has essentially the same time scale as fluorescence, in other words it is a nanosecond event.

Sample Preparation

The majority of phosphorescence measurements are carried out in rigid media at the temperature of liquid nitrogen and clearly the selection of the right solvent plays an important part in obtaining accurate measurements. The criteria for solvent selection are:

- good solubility of the analyte
- formation of a clear rigid glass at 77 K
- low phosphorescence background (high purity)

Unfortunately relatively few solvents form clear solids at 77 K. For polar compounds, ethanol is an excellent solvent and small quantities of base or acid may be added to produce a clear solid. For non-polar compounds, the most popular solvent is a mixture of diethyl ether, isopentane and ethanol in the ratio of 5 : 5 : 2 respectively, called EPA. Other solvents used are, hexane, isopentane and heptane, though even the spectrograde quality require distillation to reduce the background phosphorescence signal.

The samples are placed in a narrow quartz tube (internal diameter varying from 1 to 3 mm). The dimensions are a compromise between too small a diameter, which would give poor signal response and high scatter, and too large a diameter, which would lead to poor solidification and cracking of the glass. The sample tubes are placed in liquid nitrogen held in a quartz Dewar flask, and the latter placed in the sample holder known as a phosphoroscope.

As mentioned earlier, phosphorescence can also be observed from solid samples at room temperature, and the compounds can be divided into two types. The first includes inorganic salts and oxides such as the rare earths, europium, and uranium, which phosphoresce naturally without any sample pre-treatment. Phosphorescence is observed from these compounds by holding them in a powder holder of the solid sample accessory fitted to the PerkinElmer Model LS-5.

The second type of compounds are those which exhibit phosphorescence when adsorbed onto certain substrates such as paper, cellulose, silica, etc. Polar organic molecules when absorbed onto filter paper from solutions containing 1 N NaOH and thoroughly dried, exhibit phosphorescence. Further studies have shown that the phosphorescence could be enhanced by the addition of heavy atoms, for example iodine, silver, lead. Figure 3 shows the phosphorescence spectrum of salicylic acid adsorbed onto a paper surface from a solution containing 1 N sodium hydroxide and 1 N sodium iodide.

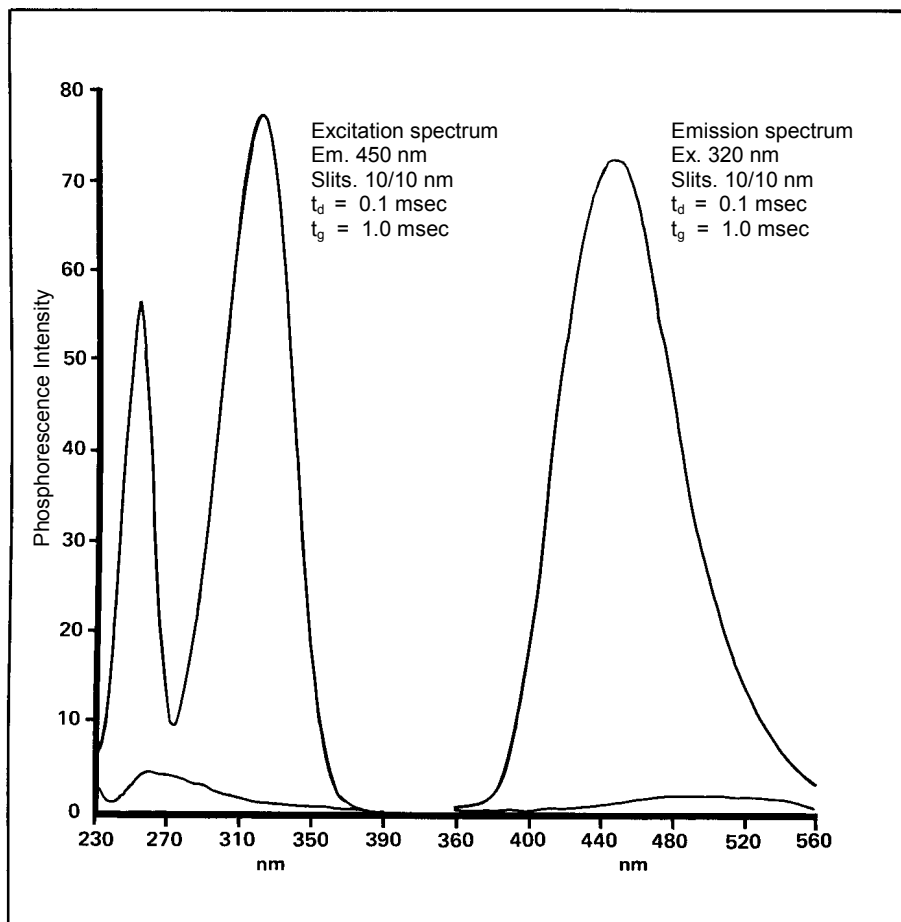


Figure 3 Room temperature phosphorescence of salicylic acid adsorbed onto paper

Sample Measurement

Two types of phosphoroscopes are used to measure phosphorescence. The first type is known as the rotating disk phosphoroscope which is used in the PerkinElmer Model 650 Fluorescence Spectrometer series. A rotating disk excitation optical chopper with three open and three larger opaque areas is used to alternately excite the sample, and allow phosphorescence to be measured. By measuring the phosphorescence intensity at several time intervals along the emission decay curve, a recorder trace of the decay with respect to time can be produced. The analytical precision and accuracy for quantitative measurements is improved by rotating the sample tube. This minimizes variation in the signal due to sample inhomogeneity resulting from imperfect glass formation at low temperatures [4, 5].

Phosphorescence spectra have been observed from "snows", in other words, aqueous solutions which have not frozen correctly, using this technique. Figure 4 shows the excitation and emission spectra of a number of halogenated biphenyls at 77 K run on a PerkinElmer Model 650-10S. The lifetime range from 3 to 430 msec in going from the iodo to the chloro derivative respectively.

The second type is the rotating can phosphoroscope, which is used in the PerkinElmer Model MPF Fluorescence Spectrometer series. Radiation from the excitation monochromator passes onto the rotating can and, when the window in the can is aligned with the excitation beam, radiation falls on the sample and excites both fluorescence and phosphorescence. The emission monochromator is blocked by the can so that fluorescence and scattered radiation cannot be detected. As the can rotates the excitation beam is blocked and the fluorescence and scattered radiation decay to a negligible amount. Further rotation of the can will bring the window into alignment with the emission monochromator entrance slit and phosphorescence radiation will pass into the emission monochromator and onto the sample photomultiplier. The rotating can or chopper rotates at speeds greater than 1000 rpm.

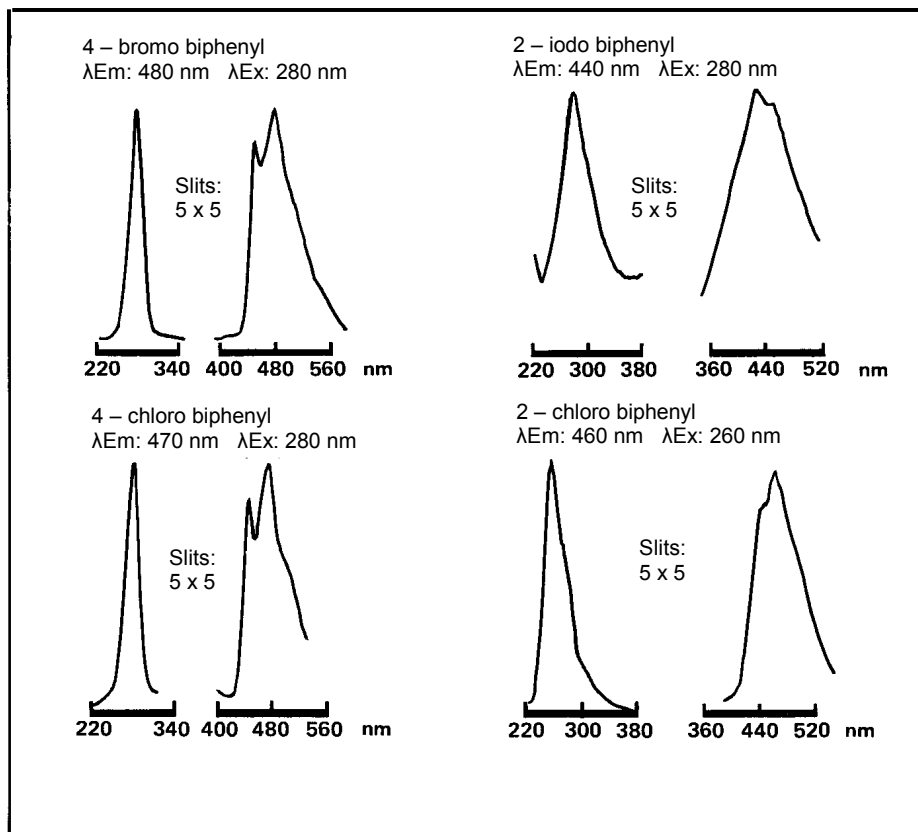


Figure 4 Low temperature phosphorescence spectra of halogenated biphenyls. Courtesy T.J. Porro

Pulsed source-time resolved phosphorimetry was first proposed by Fisher and Winefordner in 1972 [6] and with the introduction of the PerkinElmer Model LS-5 luminescence spectrometer the advantages of this system compared with the conventional mechanical chopper are realized. These advantages are as follows:

- A pulsed source produces higher peak intensities than a continuously operated xenon lamp resulting in a greater peak phosphorescence emission intensity.
- Pulsed source phosphorimetry has the advantage of time resolution compared with a mechanically modulated system permitting the analysis of organic phosphors with short lifetimes (0.1 to 50 msec) [7].

Figure 5 shows the schematic diagram of the events occurring during the excitation of a sample with the pulsed xenon source in the phosphorescence mode. The xenon lamp produces a burst of energy with a width at half peak intensity of less than 10 μ sec. During this period the phosphorescence intensity increases to a peak value (I_0) and then theoretically decays exponentially (in practice the shape of the decay curve may differ due to a number of factors such as solvent interaction and the presence of more than one compound). The signals from the sample photomultiplier are gated and both the delay of the start of the gate after the start of the flash (t_d), and the duration of the gate (t_g) can be varied in multiples of 10 μ sec. Timing is performed by a crystal clock and is highly accurate. During the cycle a quantum-corrected reference photomultiplier is used to monitor the flash intensity and the signals from the sample and reference photomultiplier are ratioed to compensate for any source instability and to provide corrected excitation spectra.

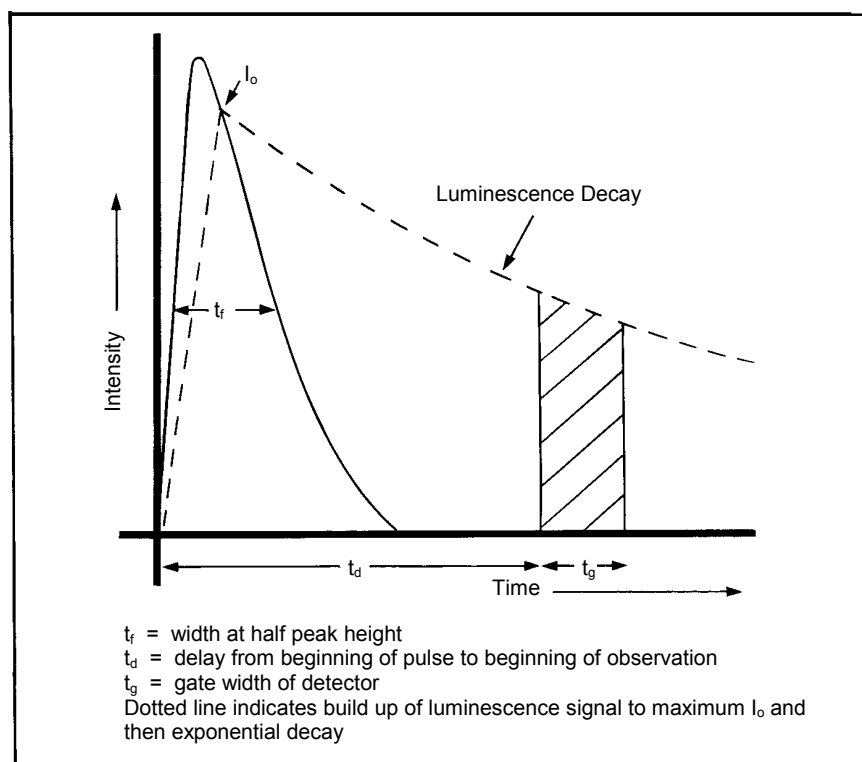


Figure 5 Schematic diagram of events occurring during the excitation of a sample with a pulsed xenon source in phosphorescence mode

Sample Handling

Although the vast majority of phosphorescence measurements are made with phosphoroscopes and solid sample holder, recent work has shown that phosphorimetry can be successfully combined with HPLC and TLC.

Lloyd [8] described packing a flow cell with a dry mixture of lint and crushed quartz to determine various enzyme compounds in an aqueous free solvent.

The earlier studies involved scraping the sample from the TLC plate, dissolving in a suitable solvent and measuring the phosphorescence at 77 K [9]. In 1975 Giffard showed that phosphorescence could be measured from a flexible TLC plate at 77 K in a modified phosphoroscope [10]. The method has been successfully applied to the measurement of phenothiazines, thiobarbiturates, and phenytoin and to analyze 6-mercaptopurine and its metabolites in blood serum. The technique can also be applied to room temperature phosphorescence [11].

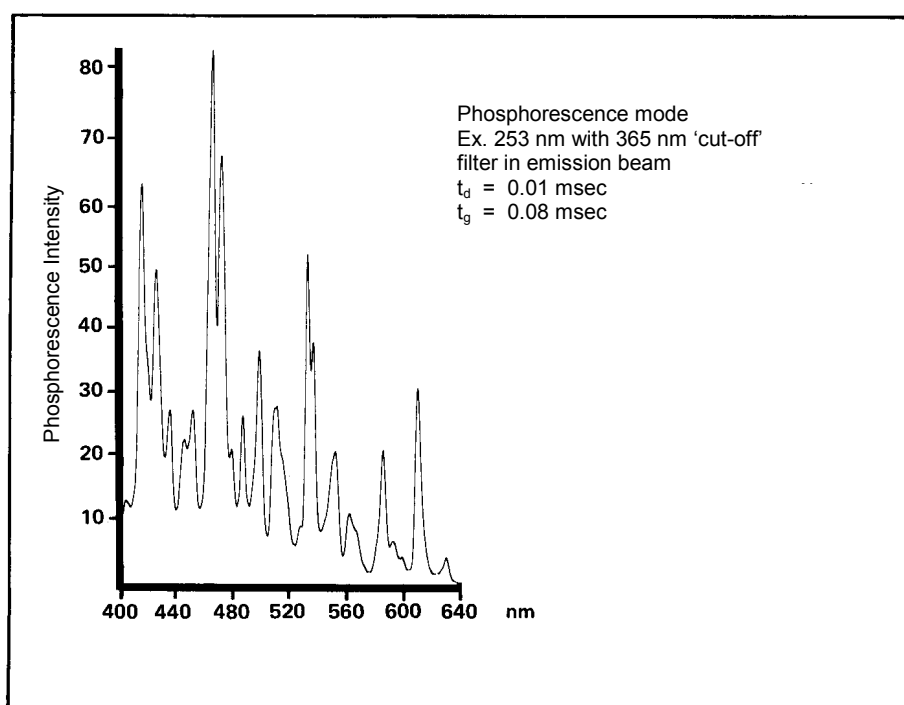


Figure 6 Phosphorescence spectrum of Eu/Y₂O₃ 0.54 mol%

Figure 6 shows the emission spectrum of a Eu:Y₂O₃ mixture measured on a PerkinElmer Model LS-5 using a solid sampling accessory. The lifetime of the various transitions can be easily measured by monitoring the intensity with varying delay times (t_d) and calculating the slope of the line obtained by plotting log intensity against time. Figure 7 shows the results of the two such plots taken at different wavelengths.

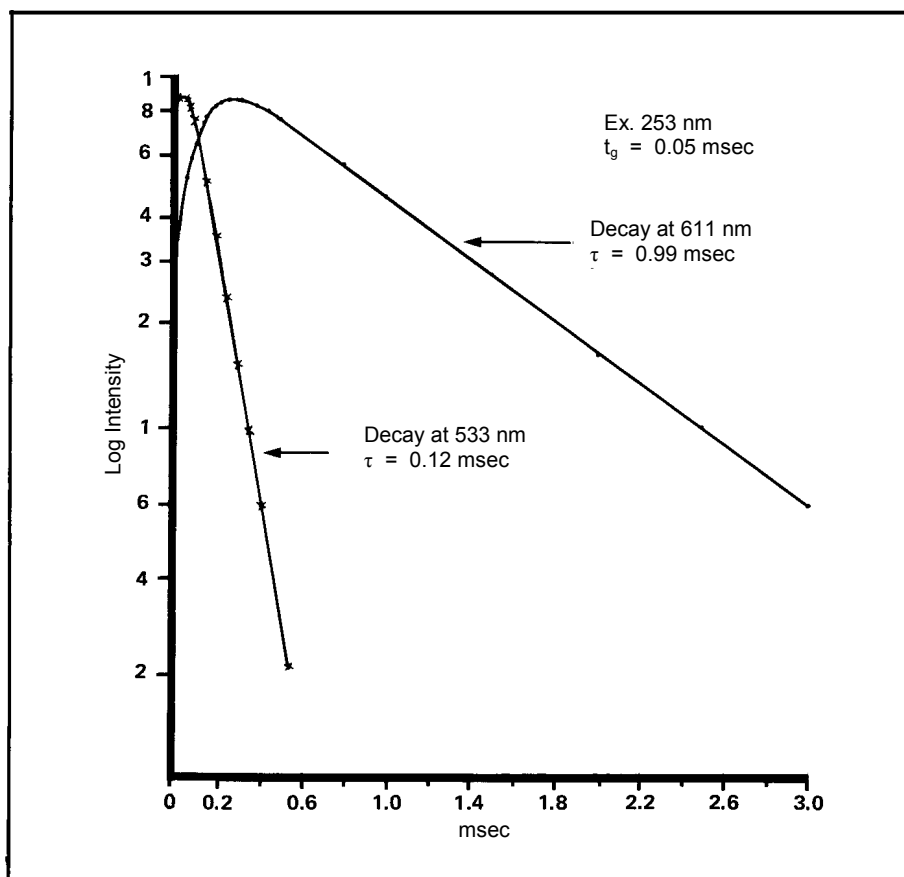


Figure 7 Plot of log intensity against time in msec for 0.54 mol% Eu in Y₂O₃

The added dimension of time can be used to discriminate between background fluorescence and Rayleigh and Raman scattering as shown by Figure 8. In the fluorescence mode the LS-5 gives an emission spectrum which contains the Rayleigh and Raman scatter superimposed upon the emission peaks of the uranyl phosphate. When measured in the phosphorescence mode, the scatter is not observed and the true emission spectrum of the uranyl ion is seen.

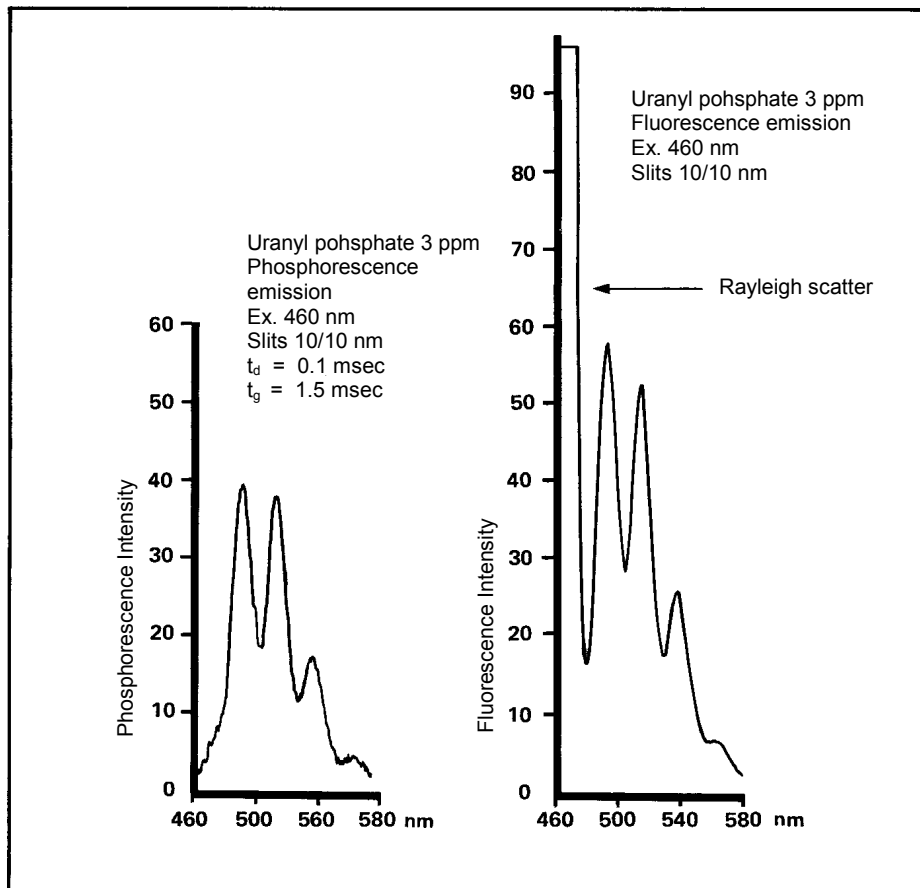


Figure 8 Discrimination between phosphorescence and Rayleigh and Raman scatter

Time can also be used to discriminate between two phosphors each with differing lifetimes. Figure 9 shows two emission spectra of a 50:50 mixture of europium and terbium chloride taken at different delay times. Europium decays with a lifetime of 0.25 msec whereas terbium has a lifetime of 0.74 msec. Setting the delay to 1 msec gives an emission spectrum which is predominantly that of terbium. Setting a short delay and sampling or gating time will have the opposite effect and favor the quickly decaying europium, inhibiting the terbium from accumulating a significant signal.

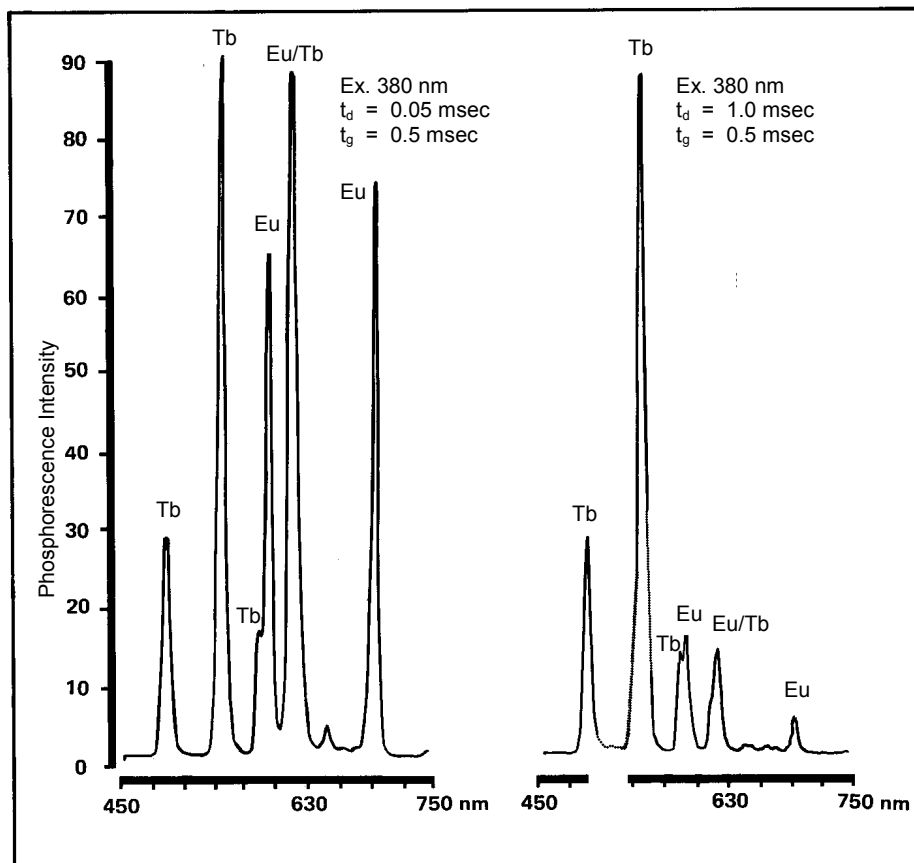


Figure 9 Emission spectrum Tb Cl₃/EuCl₃ 50/50 mixture, phosphorescence mode

Applications

The majority of phosphorescence applications have been applied in the drug and pharmaceutical field [12] and in the analysis of pesticides [13]. A number of the sulphonamide class of drugs exhibit phosphorescence as do phenobarbital, cocaine, procaine, chlorpromazine and salicylic acid. Winefordner and Tin [14] have published a paper on the determination of a number of drugs in urine and blood. Phosphorescence has been used in the detection of air and water borne pollutants [15], for the analysis of impurities in polycyclic aromatic hydrocarbons [16] and in petroleum products [17].

An On-line analysis by RTP was proposed by Vo-Dinh [18] using a continuous filter paper device. He also suggested using heavy atoms to aid the analysis of complex mixtures by RTP, as did Meyers for the determination of tryptophan and tyrosine [19]. A review on the use of phosphor coated stamps and envelopes has appeared which gives a fascinating insight into the practical use of phosphorescence [20]. The rare earths and uranyl elements phosphoresce and a number of them, particularly europium and terbium, are used as phosphors in lamps and TV tubes. The phosphorescence intensity of the rare earths increases tremendously when they are covalently bound to certain molecules and this feature has been used in the analysis of transferrin in blood [21]. Time-resolved spectroscopy offers an ideal way of removing unwanted background fluorescence with a consequent increase in sensitivity [22, 23].

For further applications and references the reader is referred to the excellent reviews which appear annually in *Analytical Chemistry*. The following table lists the phosphorescence data taken from the literature for a number of organic compounds measured at 77 K and at room temperature.

Conclusion

Although the applications of phosphorescence have been somewhat limited in the past, the introduction of new instrumentation and the recent advances made in room temperature phosphorescence should lead to an increase in its use, particularly in clinical chemistry [46] and the forensic, environmental and pharmaceutical fields. The following table gives phosphorescence data for some organic compounds.

NOTE: *Ex (excitation) and Em (emission) wavelengths are given in nm for maximum excitation and emission. Various solvents are used and the abbreviations are EtOH = ethanol; EPA = diethylether, isopentane, ethanol in the ratio 5 : 5 : 2; WM = water, methanol in the ratio 9 : 1; WE = water, ethanol in the ratio 9 : 1. The abbreviation RTP indicates room temperature phosphorescence carried out on various support materials. Detection limits are quoted in concentration units, $\mu\text{g/ml}$, but in the case of RTP measurements the detection limit is in ng total weight.*

	Compound	Solvent	Wavelengths		Life-time	Limit of Detection	Ref.
			EX	EM	τ in sec	$\mu\text{g/ml}$	
1	Acenaphthene	EtOH	300	515	-	0.2	24
2	Adenine	WM	278	406	2.9	0.02	25
		RTP	290	470	-	4.1	26
3	Adenosine	EtOH	280	422	0.8	3.2	27
4	p-Aminobenzoic acid	EtOH	305	425	-	0.001	28
5	6-Amino-6-methyl mercaptopurine	WM	321	456	0.66	0.0002	28
6	Anthracene	EtOH	300	462	-	0.05	24
7	Apomorphine HCl	EtOH	320	470	3.1	0.001	29

	Compound	Solvent	Wavelengths		Life-time	Limit of Detection	Ref.
			EX	EM	τ in sec	$\mu\text{g/ml}$	
8	Aspirin	EPA	240	380	2.1	0.10	30
9	Atropine	EtOH	360	410	1.4	0.10	14
10	1,2- Benzanthracene	EtOH	310	510	2.2	0.03	24
11	Benzimidazole	EtOH	280	406	2.3	0.006	27
12	Benzocaine	EtOH	310	430	5.3	0.007	24
13	Benzoic acid	EPA	240	400	2.4	0.005	14
14	3,4-Benzpyrene	EtOH	325	508	-	3.0	24
15	Biphenyl	EtOH	270	385	1.0	0.004	3.1
16	6-Bromopurine	WM	273	420	0.5	0.002	25
17	Brucine	EtOH	305	435	0.9	0.1	29
18	Caffeine	EtOH	285	440	2.0	0.2	14
19	Carbazole	EtOH	341	436	7.8	0.001	31
20	Chloroneb	WE	303	487	0.009	0.4	32
21	p-Chlorophenol	EtOH	290	505	<0.2	0.2	13
22	o-Chlorophenoxyacetic acid	EtOH	280	518	0.7	0.2	33
23	p-Chlorophenoxyacetic acid	EtOH	283	396	<0.5	0.004	33
24	6-Chloropurine	WM	273	419	0.64	0.002	25
		RTP	290	460	-	0.04	34
25	Chlorpromazine HCl	EtOH	320	490	0.07	0.03	14

	Compound	Solvent	Wavelengths		Life-time	Limit of Detection	Ref.
			EX	EM	τ in sec	$\mu\text{g/ml}$	
26	Chlortetracycline	EtOH	280	410	2.7	0.05	14
27	Cocaine HCl	EtOH	240	400	2.7	0.01	14
		RTP	285	460	-	0.04	36
28	Codeine	EtOH	270	505	0.3	0.01	29
29	Co-Ral	EtOH	335	510	<0.2	0.004	13
30	DDT	EtOH	270	420	0.2	0.007	13
31	Diacetylsulphanamide	EtOH	280	405	1.3	0.001	35
32	1,2,5,6-Dibenzanthracene	EtOH	340	550	1.3	0.003	24
33	Dichloran	WE	322	504	-	0.001	32
34	Dichlorophen	WE	290	487	0.028	0.1	32
		RTP	309	489	-	5.0	32
35	2,4-Dichlorophenoxyacetic acid	EtOH	289	490	<0.5	0.002	33
36	Dicumarol	EtOH	305	475	0.6	0.001	36
37	3,4-Dihydroxymandelic acid	EtOH	260	397	<0.5	0.09	37
38	3,4-Dihydroxyphenylacetic acid	EtOH	295	430	0.9	0.2	37
39	2,5-Dimethoxy-4-methyl-amphetamine	WM	289	411	3.9	0.01	38
40	N,N-Dimethyl-tryptamine	WM	286	434	6.9	0.015	38

	Compound	Solvent	Wavelengths		Life-time τ in sec	Limit of Detection $\mu\text{g/ml}$	Ref.
			EX	EM			
41	Dopa	EtOH	286	435	0.9	0.1	37
42	Dopamine	EtOH	273	410	0.9	0.15	37
43	Ephedrine	EtOH	225	390	3.6	0.2	14
44	Epinephrine	EtOH	260	412	0.6	0.2	37
45	Estradiol	EtOH	292	403	2.0	0.3	27
46	Ethyl-3-indoleacetate	EtOH	290	440	3.3	0.02	33
47	Folic acid	EtOH	367	425	-	0.004	28
48	Folpet	WE	305	440	0.6	0.001	32
49	Fluoranthene	RTP	365	545	-	0.05	39
50	Guthion	EtOH	325	420	0.6	0.06	13
51	Homovanillic acid	EtOH	292	418	1.1	0.04	37
52	5-Hydroxy indole acetic acid	RTP	312	510	-	0.5	32
53	5-Hydroxy tryptophan	RTP	320	500	-	0.5	32
54	Ibocaine HCl	WM	292	430	8.6	0.01	38
55	Indole-3-acetic acid	EtOH	290	438	<0.5	0.02	33
56	Kelthane	EtOH	285	515	<0.2	0.0006	13
57	Lidocaine	EtOH	265	400	1.1	1.2	14
58	Lysergic acid diethylamide (LSD)	WM	311	512	0.004	0.008	38

	Compound	Solvent	Wavelengths		Life-time	Limit of Detection	Ref.
			EX	EM	τ in sec	$\mu\text{g/ml}$	
59	Metanephrine	EtOH	288	418	1.3	0.02	37
60	Methoxychlor	EtOH	275	380	0.7	0.0004	33
61	6-Methylmercaptapurine	WM	291	420	0.6	0.006	25
		RTP	290	458	-	0.2	26
62	6-Methylpurine	WM	272	405	3.2	0.01	25
		RTP	268	449	-	5	40
63	Morphine	EtOH	285	500	0.3	0.01	30
64	Morphine sulphate	EtOH	265	460	0.8	10.0	30
65	Nalidixic acid	WM	336	472	0.25	12	11
		RTP	338	438	0.83	0.06	11
66	Naphthalene	EPA	310	475	1.8	0.7	41
67	β -Naphthalene acetic acid	EtOH	295	510	2.8	0.0004	33
68	β -Naphthalene sulphonic acid	RTP	286	511	-	0.2	42
69	α -Naphthol	EtOH	320	475	1.15	0.0002	13
70	β -Naphtho xyacetic acid	EtOH	328	497	2.6	0.006	33
71	Niacinamide	EtOH	270	410	-	0.2	28
72	Nicotine	EtOH	270	390	5.2	0.01	13
73	4-Nitrophenol	EtOH	355	520	<0.2	0.00002	13
74	Norepinephrine	EtOH	284	386	0.5	0.15	37

	Compound	Solvent	Wavelengths		Life-time	Limit of Detection	Ref.
			EX	EM	τ in sec	$\mu\text{g/ml}$	
75	Normetanephrine	EtOH	290	416	0.65	0.02	37
76	Papavarine HCl	EtOH	260	480	1.5	0.0005	30
77	Parathion	EtOH	360	515	<0.2	0.008	13
78	Phenacetin	EPA	360	410	-	0.2	31
79	Phenanthrene	EPA	340	465	2.6	1.0	43
80	Phenobarbital	EtOH	240	380	1.8	0.1	14
81	Phthalylsulphathiazole	EtOH	305	405	0.9	1.0	35
82	Procaine HCl	EtOH	310	430	3.5	0.01	14
83	Purine	WM	272	405	2.2	0.01	25
84	Pyrene	RTP	330	600	-	1.3	26
85	Pyridine	EtOH	310	440	1.4	0.0001	35
86	Pyridoxine HCl	EtOH	291	425	-	0.008	28
87	Quercetin	EtOH	343	480	2.1	0.3	27
88	Quinidine sulphate	EtOH	340	500	1.3	0.04	14
89	Quinine	EtOH	340	500	1.3	0.04	14
90	Quinoline	RTP	320	510	-	25	25
91	Ronnel	EtOH	300	475	<0.2	0.0006	13
92	Salicylic acid	EtOH	315	430	6.2	0.05	35
		RTP	320	470	-	0.7	26

	Compound	Solvent	Wavelengths		Life-time	Limit of Detection	Ref.
			EX	EM	τ in sec	$\mu\text{g/ml}$	
93	Serotonin	EtOH		410	-	50	44
94	Sevin	EtOH	300	510	2.0	0.004	13
95	Sodium sulphathiazole	EtOH	315	410	1.4	1.0	35
96	Strychnine phosphate	EtOH	290	440	1.2	0.05	30
97	Sulphabenzamide	EtOH	305	405	0.7	0.0001	45
98	Sulphacetamide	EtOH	280	410	1.3	0.0001	45
99	Sulphaguanidine	RTP	267	426	-	0.9	40
100	Sulphamerazine	EtOH	280	405	0.7	0.0001	45
101	Sulphamethazine	EtOH	280	410	0.8	0.0001	45
102	Sulphanilamide	EtOH	297	411	2.9	0.012	27
		RTP	267	426	-	3	26
103	Sulphapyridine	EtOH	310	440	1.4	0.0001	45
104	Sulphathiazole	EtOH	310	420	0.9	1.0	45
705	2-Thiouracil	EtOH	312	432	<0.5	0.0038	27
106	α -Tocopherol	EtOH	296	430	-	0.05	28
107	2,4,5-Trichlorophenol	WE	305	480	0.07	0.3	32
108	2,4,6-Trichlorophenol	WE	297	482	0.06	0.16	32
109	Tryptophan	EtOH	295	440	1.5	0.002	35
		RTP	280	448	-	4	26

	Compound	Solvent	Wavelengths		Life-time	Limit of Detection	Ref.
			EX	EM	τ in sec	$\mu\text{g/ml}$	
110	Tyrosine	EtOH	253	394	1.9	0.02	28
111	Vanillin	EtOH	332	519	-	0.1	40
112	Warfarin	EtOH	305	460	0.8	0.01	37
113	Yohimbine HCl	EtOH	290	410	7.4	0.01	29

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