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Low- and room-temperature phosphorescence characteristics of some pharmaceutical compounds

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Abstract: Phosphorescence spectral characteristics, lifetimes and limits of detection of 30 pharmaceutical compounds are examined. A particular study on eight phenothiazine derivatives is carried out. Low and room temperature data obtained using an ethanol:water 80:20 glass substrate and a filter paper substrate, respectively, are compared. Lifetimes are corrected for the time constant of the measurement circuit.

Keywords: *Low temperature phosphorescence; room temperature phosphorescence; phenothiazine derivatives.*

Introduction

Analysis of drugs by conventional low temperature phosphorimetry (LTP) has been shown to be a sensitive and selective technique [1–6]. However, this method has been rarely used in biological and clinical applications because of the need for cryogenic equipment, the problems associated with the introduction of the sample into the cryogenic system (handling and cooling rate of the sample) and because of solvent restrictions.

In the past decade, room temperature phosphorimetry (RTP) has received considerable attention in drug analysis because it is simpler compared to LTP, cryogenic equipment is not needed, no difficulties are encountered with cracked glasses or snowed matrices and only a small amount of sample is required. Also, because fewer compounds phosphoresce at room temperature compared to low temperature, the selectivity of the technique is increased. Nevertheless, three drawbacks exist: higher (poorer) limits of detection (LODs) generally result for RTP than LTP; all substrates used up to now show a broad background phosphorescence band; and an appropriate time-consuming drying step is required. Among the different solid substrates which have been used at room temperature, filter paper has been the most popular because phosphorescence is induced in a wide variety of samples and it is inexpensive and readily available.

Bower *et al.* [7] reported spectral data and limits of detection for several

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pharmaceuticals measured by RTP under various experimental conditions. Later, RTP has been used successfully for the analysis of drugs in pharmaceutical formulations [8–11] and in human serum [11]. The selectivity factor in RTP is advantageous because the binders and additives used in tablet formulations usually do not phosphoresce under the experimental conditions commonly applied, and the same can be said of the components normally present in human serum. Gifford *et al.* [12] have also studied the low temperature phosphorescence characteristics of phenothiazine derivatives.

Furthermore, one of the advantages of phosphorimetry is the possibility of distinguishing between phosphors with overlapping spectra but with different lifetimes. Therefore, lifetime data are useful in devising time-resolved phosphorimetric procedures of analysis. Previously, Harbaugh *et al.* [13, 14] measured phosphorescence lifetimes and assayed drug mixtures by time-resolved phosphorimetry.

In this paper, the LTP and RTP spectral characteristics, lifetimes and limits of detection of 30 pharmaceuticals are examined, in order to evaluate the potential analytical utility of these techniques for the detection and determination of these compounds in real analytical situations.

Experimental

Reagents

The drugs studied are listed in Table 1. These drugs were used as received from the manufacturers without any recrystallisation or purification. Ethanol was also used without further purification. Barnstead nanopure deionised water (Barnstead Sybron Corp., Boston, MA) was used throughout the study. All glassware was cleaned with Micro cleaning solution (International Products Corp., Trenton, NJ) by means of an ultrasonic cleaning system (Cole Palmer, Chicago, IL) for 20 min, rinsed three times with distilled water and three times with Barnstead deionised water.

Standard stock solutions were prepared by dissolving in a 80:20 ethanol:water mixture an accurately weighed amount of each drug substance. The stock solutions were stored in amberglass bottles in a refrigerator to minimise decomposition. The working solutions were prepared from the stock solutions by serial dilutions.

Apparatus and procedure

Phosphorescence was measured with an Aminco–Bowman spectrophotofluorimeter provided with a 150 W xenon arc lamp. An Aminco ratio photometer supplied high voltage to a Hamamatsu 1P21 photomultiplier tube. Spectra were obtained using a Fisher Recordall Series 5000 recorder and were uncorrected for instrumental response. A rotating can phosphoroscope designed in our laboratory was used [6]. It was operated by a small motor connected to a variable Staco type 2PF 1010 autotransformer (Staco Inc., Dayton, OH). The sample compartment had a circular opening at the top to accommodate the low temperature and room temperature sample holders. The instrumental conditions were as follows: monochromator slits, 1 mm, emission spectra scan rate, 220 nm/min and excitation spectra scan rate, 270 nm/min.

The speed of rotation of the phosphoroscope, measured with a storage oscilloscope, was 350 Hz. Taking into account the position and size of the windows, there was about 0.5 ms between the end of the excitation period and the beginning of the measurement period. Therefore, phosphors with lifetimes of the order of 0.5 ms or less were measured with a loss of sensitivity.

Low temperature measurements

A standard Dewar flask with a quartz optical tip was held in place by a ring slip-fitted into the compartment opening. A cylindrical cover was then fitted over the Dewar flask onto the upper part of the sample compartment. The quartz sample tube, sealed at one end, was 25 cm long, 5 mm in external diameter and 3 mm in internal diameter. The sample tube was immersed into the liquid nitrogen-filled Dewar flask and held rigidly in place. The volume of sample used was about 150 μl, while the illuminated volume was about 50 μl. The sample tube was rinsed three times with the sample solution before each measurement. To avoid the formation of frost on the Dewar flask optical tip, a stream of dry nitrogen was passed continuously through the sample compartment. Nitrogen gas was dried using a laboratory gas drying unit filled with anhydrous calcium sulphate (W. A. Hammond Drierite Co., Xenia, OH).

Room temperature measurements

A finger-type sample holder was used, which was a modification of one described before [15]. The holder tip consisted of a cylindrical aluminium block (12 mm diameter, 33 mm long) with the lower half of the tip milled to be semicircular. A slit plate (15 mm long, 11 mm wide and 1.5 mm thick) with a 6-mm diameter hole in the centre served to hold each paper disc in place by means of two screws. The tip was screwed to a 25-cm long rod which was firmly attached to a cylindrical cover. The entire assembly was painted black to reduce the scattered light from the shiny metal surfaces. To avoid positioning problems which would lead to a lack of precision between samples, the same tip was used throughout all experiments and was never removed from the sample holder.

Whatman No. 1 filter paper was chosen as the substrate and was used as supplied. Filter paper circles, 10 mm diameter, were cut with a paper hole puncher. The paper circles were placed on the sample holder tip with tweezers and spotted alternatively with 5 μl aliquots of the analyte and blank solutions by means of a Hamilton microsyringe. Immediately afterwards the sample holder was inserted into the sample compartment where the sample was allowed to dry for about 20 min under a flow of dry nitrogen. A series of experiments carried out using previous heating of the paper in an oven at 40°C for periods of 5–20 min gave rise to poorer results, probably because of decomposition of the analytes. Similarly, the use of an infrared lamp to dry samples led to a loss of sensitivity.

Results and Discussion

Low temperature phosphorescence spectral characteristics, limits of detection and relative standard deviations are reported in Table 1. Room temperature phosphorescence data are shown in Table 2. Compounds examined for which no phosphorescence signal was detectable at a concentration larger than 500 μg/ml at low and room temperature were alphaprodine, cycrimine, ergotamine, mesantoin, methapyrilene, nortriptyline, nylidrin, persantine, phendimetrazine, propoxyphene and tranylcypromine (the last compound was found to be phosphorescent at 77K by Gifford *et al.* [16]). The absence of any measurable signal may be a result of instrumental limitations caused by the very short-lived phosphors (less than 0.5 ms).

It can be seen that only some of the compounds which phosphoresced in ethanolic glasses at 77K, also phosphoresced on filter paper at room temperature; this accounts for the greater selectivity of RTP over LTP.

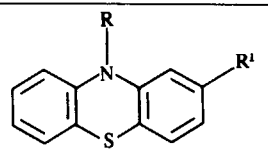
Table 1
Analytical figures of merit for several drugs using low-temperature phosphorimetry

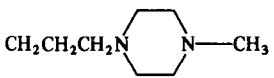
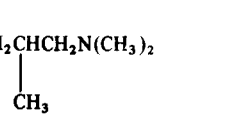
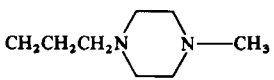
Compound	Excitation λ_{ex} (nm)	Spectral half-width (nm)	Emission λ_{em} (nm)	Spectral half-width (nm)	LOD (ng/ml)*	% RSD†	Lifetime (s)
Bendroflumethiazide‡	243, 289§	49, 53	437	63	9.5 (0.48)	1.9 (0.15)	‖
Chlorpheniramine	237, 268 304	55, 58 56	423	107	134 (6.7)*	6.2 (1.0)	‖
Chlorpromazine‡	273, 326	25, 50	496	66	8.4 (0.42)	6.4 (0.2)	‖
Desipramine	296	88	431	86	127 (6.4)	1.3 (1.0)	‖
Diethylpropion	268, 312 Similar intensities	65, 51	430	80	2.3 (0.12)**	1.1 (0.05)	1.28 ± 0.15
Diphenylpyraline	239	66	384	92	4.1 (0.20)	0.8 (0.5)	4.02 ± 0.07
Fentanyl	278	35	391	85	39 (2.0)	0.9 (10)	2.49 ± 0.12
Glutethimide	278	54	385	92	9.5 (0.48)	0.9 (1)	3.90 ± 0.09
Ibogaine	246, 305	67, 57	429	66	4.6 (0.23)	1.70 (0.1)	1.78 ± 0.14

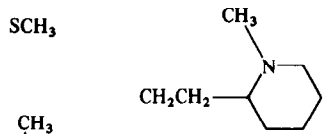
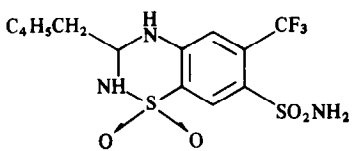
Phencyclidine	285	54	404	94	3.8 (0.19)	0.7 (1)	5.05 ± 0.08
Phenoxybenzamine	234, 293	76, 79	401	81	30 (1.5)	1.5 (1)	1.30 ± 0.09
Prochlorperazine‡	263, 314	39, 49	487	75	2.7 (0.14)	3.3 (0.1)	‖
Promazine‡	259, 317	32, 40	490	76	5.3 (0.26)	2.5 (0.1)	‖
Serentil‡	270, 323	39, 67	516	89	2.6 (0.13)	1.1 (0.1)	‖
Thioridazine‡	268, 316	29, 43	481	66	3.9 (0.20)	0.7 (0.15)	‖
Trifluoperazine‡	263, 307	34, 65	500	87	0.8 (0.04)	3.6 (0.1)	‖
Triflupromazine‡	266, 313	28, 54	499	66	0.4 (0.002)	2.6 (0.025)	‖
Trimeprazine‡	259, 311	31, 43	482	67	0.9 (0.045)	0.9 (0.1)	‖

*Absolute LOD in ng is given in parentheses. Values were calculated based on 12 blank measurements.
†In parentheses, concentration levels in μg/ml.
‡Structure is shown in Table 2.
§Most intense peak is underlined.
‖Lifetime below 0.2 s.
**Excitation wavelength is 268 nm.

Table 2
Analytical figures of merit of several drugs using room temperature phosphorimetry*



Compound	R^1	R	Excitation λ_{ex} (nm)	Spectral half-width (nm)	Emission λ_{em} (nm)	Spectral half-width (nm)	$\Delta\lambda_{ex}$† (nm)	LOD‡ (ng)	% RSD§
Chlorpromazine	Cl	$CH_2CH_2CH_2N(CH_3)_2$	264,‖ 324	32, 69	512	75	−9, −2	52	1.4
Prochlorperazine	Cl		257, 312	27, 72	519	94	−6, −2	73	1.3
Promazine	H	$CH_2CH_2CH_2N(CH_3)_2$	263, 324	33, 65	518	77	4, 7	3	1.1
Trimeprazine	H		261, 317	27, 65	507	77	2, 6	19	2.4
Triflupromazine	CF_3	$CH_2CH_2CH_2N(CH_3)_2$	271, 329	36, 71	516	89	5, 16	39	2.1
Trifluoperazine	CF_3		270, 325	32, 63	523	102	7, 18	104	3.5

Thiridazine		272, 319	43, 65	511	92	4, 3	15	2.4
Serentil		283, 337	42, 72	532	86	12, 14	19	3.3
Bendroflumethiazide		279, ~314(sh)	63	494	110	36, 25	72	2.4

*Lifetimes were shorter than 0.2 s in all cases.
†Excitation spectral shifts between RTP and LTP, $\Delta\lambda_{ex} = \lambda_{ex}$ (RTP) $- \lambda_{ex}$ (LTP).
‡Values were calculated based on 12 blank measurements.
§At a 100 ppm concentration level in all cases.
‖Most intense peak is underlined.

Eight of the nine compounds examined which exhibit phosphorescence at room temperature are phenothiazine derivatives and the structure of the ninth compound, bendroflumethiazide, has some structural similarity with the phenothiazine group and therefore, may have a similar ability to bind with the paper substrate active sites (see Table 2).

A comparative study of the phenothiazine derivative spectra show that the influence of the substituents is very small, even when the atom directly bonded to the phenothiazine rings (e.g. R' = H, Cl or C) changes. Because of the small structural differences, no spectra–structure correlations can be deduced.

However, if the spectra at low and room temperature are compared, a certain spectra–structure relationship appears as is evident from the results in Table 2. Only derivatives with R' = Cl show RTP blue-shifts with respect to the LTP excitation spectra. The other derivatives (R' = H or C) show red-shifts, which become somewhat larger as the molecules become more polar. This could be related to their ability to bind to the active sites on the paper surface.

On the other hand, phosphorescence emission spectra do not lead to obvious relationships, because all RTP spectra are red-shifted and the bands are broader compared to low temperature spectra; this has been observed before with other compounds [15, 17–21]. The excitation and emission spectra of promazine at low and room temperature are shown in Fig. 1.

The limit of detection was defined as the concentration (LTP) or weight (RTP) of analyte giving a signal three times the standard deviation of the blank solution at the maximum excitation and emission wavelengths [21]. Except for promazine, the limits of detection at room temperature are greater than 15 ng; they can be improved by using heavy-atom enhancers as well as a preconcentration technique, such as the repeated application of sample on the same paper disc. These studies are currently being done. On the other hand, relative standard deviations of the phosphorescence of the analytes are within the range 1.1–3.5% for RTP and between 0.7 and 6.4% for LTP.

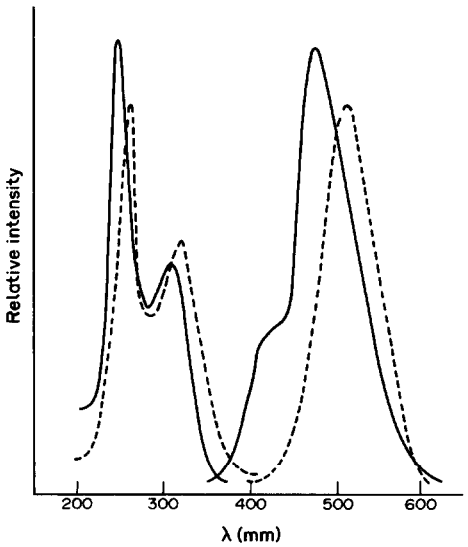


Figure 1
Phosphorescence excitation and emission spectra of promazine at low (——) and room temperature (– – –) on paper substrate.

Measurement of lifetimes

Long lifetimes (longer than the time constant or resistance–capacitance value of the measurement circuit [22] may be determined by simple recording of the decay of the signal when the exciting light is suddenly shut off. Of course, the decay curves of the phosphors (τ_{an}) must be corrected for the instrumental distortion due to the circuit time constant.

If a mirror is used in the sample position, an exponential decay curve due to the apparatus response τ_{ap}, is obtained:

$$I_t = I_o \exp(-t/\tau_{ap}) \quad (1)$$

Linearisation and regression analysis of this equation can be used to establish the value of τ_{ap}. On the other hand, the phosphor decay curve obeys a similar equation:

$$I'_t = I_o \exp(-t/\tau_{an}) \quad (2)$$

However, because of the distortion effect of the apparatus response, the recorded intensity, I'_t, differs from I_t. Assuming that the value of I'_t is also given by an exponential equation with a lifetime $\tau \simeq \tau_{an} + \tau_{ap}$ [23], the corrected lifetime of the analyte can be calculated from τ, by subtraction of τ_{ap} which is obtained from independent experiments using a mirror or even the decay curve of the blank signal, provided that it is large enough and does not contain phosphors with lifetimes of the same order as, or larger than, the analyte lifetime.

The response time, τ_{ap}, of the spectrometer–recorder assembly was measured using a mirror and recording five decay curves. Six evenly distributed points were taken from each curve, the first point being on the 95–80% intensity part of the curve and the last on the 30–20% part. Values of τ_{ap} of 1.97 ± 0.05 s and 0.40 ± 0.07 s were obtained when the spectrometer time constant was set to positions 4 and 1, respectively. The τ values for the decay curves of the analytes and blanks were obtained using the same procedure and were used to calculate the corrected τ_{an} and τ_{blank} values. In all cases, correlation coefficients higher than 0.995 were found.

The correction procedure for the apparatus response was tested using biphenyl on paper substrate. The corrected lifetimes using time constant positions 4 and 1 were 1.6 ± 0.1 s in both cases. The same value was obtained using a storage oscilloscope directly connected to the photomultiplier (PM) output. Decay curves obtained for the blank at room temperature gave values of τ_{blank} which were indistinguishable from τ_{ap}, showing that they do not contain phosphors of long lifetimes at a detectable concentration.

Lifetimes of the phosphorescent compounds at low temperature are shown in Table 1. The lifetime of 4.8 s for phencyclidine agrees with the value obtained by Boutilier *et al.* [24] in a 10:90 ethanol:water glass at 77K, using a CMX-Laser PM-tube and boxcar integration. Compounds exhibiting lifetimes shorter than 0.2 s at low and room temperature could not be measured with the Aminco–Bowman spectrometer–recorder assembly. Boutilier *et al.* [25] found a lifetime of 67 ms for thiopropazate (a phenothiazine derivative) in a 10:90 ethanol:water matrix at 77K. At room temperature, lifetimes are much shorter than at low temperature [26].

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