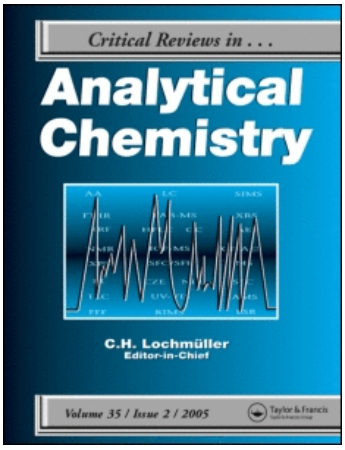

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A Review of Heavy-Atom-Induced Room-Temperature Phosphorescence: a Straightforward Phosphorimetric Method

Antonio Segura Carretero[a]; Alfonso Salinas Castillo[a]; Alberto Fernández Gutiérrez[a]
[a] Department of Analytical Chemistry, Faculty of Sciences, University of Granada, Granada, Spain

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A Review of Heavy-Atom-Induced Room-Temperature Phosphorescence: a Straightforward Phosphorimetric Method

Antonio Segura Carretero, Alfonso Salinas Castillo, and Alberto Fernández Gutiérrez

Department of Analytical Chemistry, Faculty of Sciences, University of Granada, Granada, Spain

This review discusses the development of heavy-atom-induced, room-temperature phosphorescence (HAI-RTP), a new technique based on the use of RTP emitted directly from the analyte in a fluid solution with no protective medium except the presence of high concentrations of a heavy-atom perturber. These experimental conditions permit sufficient interaction between the perturber and the analytes to produce an effective population of their triplet states and thus emit intense phosphorescence.

Keywords heavy-atom-induced, room-temperature phosphorescence

INTRODUCTION

Luminescence is a branch of spectroscopy that deals primarily with the electronic states of an atom or molecule as opposed to its vibrational, rotational, or nuclear energy states. Fluorescence and phosphorescence, both of which involve the optical detection and spectral analysis of light emitted, are the two sister techniques of luminescence spectroscopy.

The sensitivity of phosphorimetry is slightly lower than that of fluorimetry but phosphorimetry gains in that phosphorescence emission wavelengths are generally far removed from the excitation wavelength and the time scales are longer and therefore more manageable. Furthermore, the equipment needed to measure fluorescence lifetime ($\sim 10^{-8}$ s) is more sophisticated than that required to measure phosphorescence lifetime ($10^{-4} - 10$ s). Thus, the advantages of phosphorimetry for lifetime measurements mean that it can be used to study various molecular motions in biological samples and polymers that cannot be studied by fluorimetry because of the very short lifetime of fluorescence. Finally, it is possible to achieve much higher selectivity with phosphorimetry than with spectrophotometry and fluorimetry because only a few compounds phosphoresce compared to the much larger number which fluoresce or absorb radiation.

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Address correspondence to Antonio Segura Carretero, Department of Analytical Chemistry, Faculty of Sciences, University of Granada, Avda. Fuentenueva s/n, 18071, Granada, Spain. E-mail: ansegura@ugr.es

Until 1980, room-temperature phosphorescence (RTP) in solution was only observed under certain special conditions in a few compounds dissolved in organic solvents (1–4). Parker (1, 2) demonstrated for the first time that phosphorescence could be observed at room temperature with benzophenone in fluorocarbon solvents. Clark et al. (3) went on to demonstrate the possibility of obtaining phosphorescent emission with benzophenone and acetophenone in benzene and isooctane at 23°C, and in 1972 Bonner et al. (4), by comparing their results with those reported in the literature for experiments made at 77 K, confirmed the RTP in solution of biphenyls using either ethanol or alkanes as solvents. This was put down to the specific rigidity of the luminophor or the existence of a spin-forbidden transition enhancer and was regarded as a phenomenon proper to this kind of compound.

These pioneering studies were conducted mainly in the realm of physics. The phosphorimetric signal was used on a theoretical basis and considered to be of interest but no special regard was paid to its potential value in detection systems in analytical chemistry.

Phosphorescence quantum efficiency (ϕ_p) is defined as the ratio between the number of photons absorbed and the number of phosphorescence photons emitted. This is an intrinsic parameter for any compound studied experimentally (5–7). For solutions of low concentration, where photochemical reactions such as dimerization can be neglected, phosphorescence quantum efficiency is given by

$$\phi_p = \phi_{ISC} \frac{k_p}{k_P + k_{IC} + \sum_Q k_Q [Q]},$$

where ϕ_{ISC} is the intersystem-crossing quantum efficiency, k_p and k_{IC} are the first-order rate constants (s^{-1}) of the phosphorescence process and of the intramolecular radiationless deactivation of the triplet state, respectively, $k_Q[Q]$ gives the rate of deactivation by bimolecular quenching, k_q is the second-order rate constant ($l\,mol^{-1}\,s^{-1}$) (where this parameter is directly proportional to temperature and inversely proportional to the viscosity of medium), and $[Q]$ is the concentration of the quencher. For fluid solutions at room temperature, where k_Q will be in the order of $10^9 - 10^{10}$ l/mol s, $\Sigma_Q k_Q[Q]$ will be the dominant term. To obtain emission signals this term needs to be made as small as possible by diminishing $[Q]$ (dissolved molecular oxygen has to be removed almost completely) and/or diminishing k_Q (by decreasing the temperature or increasing the viscosity).

Turro (8) pointed out that if phosphorescence can be obtained at 77 K it can also usually be seen in a fluid solution at room temperature, if two conditions are fulfilled: (1) any impurities capable of quenching the triplet states are rigorously excluded; (2) the triplet state does not undergo activated unimolecular deactivation, thus having a rate constant (^3K_m) greater than or equal to 10^4 times the phosphorescence rate constant (K_p). In others words, phosphorescence can be seen at room temperature in a fluid solution provided that $^3K_m < 10^4 K_p$ and the phosphorescence quantum yield (ϕ_p) can be expressed approximately by equation based on the dynamic graph,

$$\phi_p = \frac{K_p}{{}^3K_m + K_q[q]},$$

where K_q is the rate constant for the bimolecular deactivation of the triplet state and $[q]$ the concentration of the quencher. A typical value of K_p for a molecule in an n, π^* triplet state (T_1) is 10^2 s, whilst in a π, π^* triplet state it is 10^{-1}s (13). With these K_p values, the limiting concentrations of quencher for the observation of phosphorescence can be calculated if the quenching is diffusionally controlled. For phosphorescence to be seen ϕ_p should be at least 10^{-4}, while for nonviscous, organic solvents the rate constant of diffusion (K_{dif}) is about 10^{10} l/mol s. In general it can be assumed that $K_{dif} = K_q$ and so the conditions for observing phosphorescence are: for T_1(n, π^*), $K_{dif}\,[q] < 10^6$ s, therefore $[q] < 10^{-4}$ mol/l; and for $T_1(\pi, \pi^*)$, $K_{dif}\,[q] < 10^3$ s, therefore $[q] < 10^{-7}$ mol/l.

A concentration of 10^{-4} mol/l for q can be readily obtained experimentally but a concentration of 10^{-7} mol/l is more difficult. Thus, it is easy to observe fluid RTP of compounds with T_1(n, π^*) states whereas it is quite difficult with $T_1(\pi, \pi^*)$ states. This conclusion concurs with our experimental results. For example, the NP-RTP emissions of naphthalene, anthracene phenanthrene and pyrene are very weak or cannot be seen at all because their triplet states are $T_1(\pi, \pi^*)$, in which, even in very pure solvents (impurity level including oxygen below 10^{-9} mol/l), the quantum yields of phosphorescence (ϕ_p) are in the order of 10^{-6}.

For molecules with no heteroatom present, electronic transitions generally involve the promotion of an electron from a bonding π orbital to an anti-bonding π^* one. For conjugated systems with N, O or S heteroatoms in the conjugated system or with substituents containing N, O, or S atoms, an electronic state may result from the promotion of an electron from a bonding π orbital to an antibonding π^* orbital.

Another way to increase the quantum efficiency of phosphorescence in HAI-RTP is by decreasing $[Q]$ and/or increasing intersystem crossing efficiency, ϕ_{ISC}, by adding high concentrations of a heavy-atom salt. The latter permits greater interaction with the analyte (larger number and more effective interactions, but the interaction distances are closer than with cyclodextrins, micelles or microemulsions), producing an effective population of the triplet state and thus intense phosphorescence.

Surfactants and cyclodextrins have been found to produce some very interesting photophysical phenomena and have formed the basis of several new analytical approaches. Organized aggregates have the ability to arrange reagents at the molecular level, bringing interacting species together with high specificity.

Micellar-stabilized, room-temperature phosphorescence (MS-RTP) is based on the use of micellar solutions to observe phosphorescence emitted by aromatic molecules in fluid solutions at room temperature. In a micellar solution the analytes included in the micelle are apparently protected from the quenchers present in the solution. Nevertheless any attempt to shield the solutes is ineffective due to the dynamic nature of micelles and the association–dissociation processes between the analyte and the micelles. Quenchers can either enter the micelles or may be excluded, as in the electrostatic repulsion of anionic quenchers from anionic micelles. RTP in a micellar solution usually requires the presence of a heavy atom, which is situated as a counter ion outside the micelle, thus being in proximity to the hydrophobic molecules associated with the micelle. The high local concentration of the heavy atoms produces an efficient spin-orbit coupling which can diminish fluorescence and increase phosphorescence by an additional internal heavy-atom effect. Furthermore, phosphorescence can only be observed in oxygen-free solutions, as oxygen is an effective quencher that easily penetrates into the micelles. Aqueous solutions are usually deoxygenated by bubbling nitrogen through them, but it is difficult to do this with a micellar solution because of foaming.

Microemulsions are homogeneous dispersions of very small drops of water in oil (W/O) or of oil in water (O/W) in the presence of large amounts of a surfactant and co-surfactant (usually a medium-sized alcohol with an alkyl group of C-4 to C-8). Water in oil microemulsions contain an aqueous core and are similar to inverted micelles. Oil in water microemulsions, on the other hand, contain a sizeable hydrocarbon core and are similar to normal micelles. The advantage of microemulsions compared to normal micelles is that the hydrophobic core itself is propitious for dissolving relatively high concentrations of hydrophobic molecules and also large organic molecules with dimensions approaching those of many micelles in the aggregate.

Cyclodextrins are host molecules for inclusion compounds, capable of trapping or complexing both small and large

molecules depending on their cavity sizes. The formation of an inclusion compound is similar to the dynamic solubilization of molecules in micelles. One different feature of these systems, however, is the existence of cavities of finite dimensions which necessarily leads to certain limits in the size and/or shape of guest molecules. The inclusion causes restrictions in the mobility of the molecule and/or alterations in local polarity. The main advantage derived from this is an increase in emission with extra sensitivity.

As can be seen, with these methods it is essential to provide a protective ordered medium to minimize self-quenching and to arrange the reagents at the molecular level in order to increase the proximity of the heavy atoms and analytes. Although a micellar medium is capable of dissolving apolar compounds, these organized media have several concomitant problems, including the formation of foams during the homogenization of the samples, clean-up of the flask, precipitation with different heavy atoms, and delays in the elimination of oxygen dissolved within the micelles. Added to this, most phosphorimetric methods, because they use cyclodextrins, require time-consuming deoxygenation of the samples and give rather poor detection limits. Neither can these methods be easily adapted to detection in liquid chromatography or flow-injection analysis.

New studies were carried out in order to discover whether phosphorescence could be achieved in solution without organized media. Donkerbroek et al. (9, 10) demonstrated the analytical potential of sensitized RTP in fluid solutions by using triplet-triplet energy transfer from the donor (analyte of interest) to the acceptor (biacetyl or 1,4-dibromonaphthalene).

HEAVY-ATOM-INDUCED, ROOM-TEMPERATURE PHOSPHORESCENCE

Until 1998, it was customary in phosphorimetry to use organized media (micelle, microemulsion or cyclodextrin) to increase the viscosity of the microenvironment and avoid intermolecular collision, plus heavy atoms to help intersystem crossing and deoxygenators to eliminate any dissolved oxygen.

A New Phosphorescence Method in Solution

In 1995 Li and Huang (11), at Tsing Hua University (Beijing, China), studied the emission conditions and spectral properties of 5-dimethylaminonaphthalene sulphonylchloride (DNS-Cl) and derivative products from amino acids by micelle-stabilized, room-temperature phosphorescence (MS-RTP) and solid-substrate, room-temperature phosphorescence (SS-RTP). Subsequently this research group (12, 13) confirmed that it was possible to obtain a phosphorescent signal from these compounds in aqueous solutions with no organised medium and established the phosphorescence characteristics (wavelengths, intensity and life time) for each compound (see Table 1). The authors proposed using a phosphorimetric method to determine dansyl chloride in an aqueous system by adding thallium nitrate as heavy-atom perturber and sodium sulphite as deoxygenator. They obtained satisfactory linearity between RTP intensity and the concentration of DNS-Cl in the range of $2.0\ 10^{-7}$ to $1.0\ 10^{-5}$ M, with a detection limit of $1.9\ 10^{-8}$ M.

The fluid RTP method reported is different from other kinds of fluid RTP methods developed previously, such as CD-RTP or MS-RTP, and has many advantages over these methods:

1. As it does not require a microscopically ordered protective medium the method is not only simple but also avoids the detrimental effects to the analyte frequently caused by the addition of such a medium.
2. Several important advantages to the system in question are the transparency of the solution, no precipitate (as with CD-RTP) and no foam (as with MS-RTP), together with the fact that the RTP signal can be induced directly within the aqueous phase, which makes it possible to combine with other analytical techniques such as liquid chromatography (LC), flow-injection analysis (FIA) and capillary electrophoresis (CE), and to perform automatic on-line analyses.
3. Chemical deoxygenation with Na_2SO_3 is simple and precise and may also be used to adjust the pH level of the system, thus simplifying the experimental procedure.

In 1996 the authors of this review (14) reported the determination of β-naphtoxyacetic acid using TX-100 as micellar medium at a concentration fifteen times lower than the critical micellar concentration. Two years later they confirmed that it was possible to obtain phosphorescence signals in solution by using aqueous solutions of the analytes alone in the presence of a heavy atom (KI and $TlNO_3$) and sodium sulphite as oxygen scavenger. The technique was called "heavy-atom-induced, room-temperature phosphorescence" (HAI-RTP) (15) and demonstrated this innovative analytical technique by obtaining RTP signals in solution with three naphthalene derivatives, a pharmaceutical compound (naphazoline, NPZ), a plant growth regulator (naphthoxyacetic acid, NOA) and a polycyclic aromatic hydrocarbon (acenaphthene, ACE) (see Table 1). Different heavy-atom salts were assayed (KI, NaI, KBr, NaBr, KCl, $TlNO_3$, $Pb(NO_3)_2$, and $AgNO_3$) at several concentrations. In all cases, it was proved that nonphosphorescence responses could be obtained from the analytes in the complete absence of a heavy atom while, in general, HAI-RTP intensity increased concomitantly with the heavy-atom concentration. Concentrations of 1 M for KI and 0.25 and 0.05 M for $TlNO_3$ were found to be optimum for the three compounds, NPZ, NOA, and Ace, respectively. Three analytical methods were proposed, with detection limits of 167, 44, and 65 ng/ml for β-naphthoxyacetic acid, naphazoline, and acenaphthene, respectively. This work demonstrated that this method can be used to determine derivative naphthalenes and confirmed the possibility of using another salt (KI) as heavy-atom perturber.

Li et al. (16) then reported that many naphthalene derivatives also emit RTP in aqueous solutions under similar conditions in the absence of a protective medium. Such an RTP emission was referred to as non-protected, fluid room-temperature

TABLE 1
Compounds, spectroscopic characteristics, experimental conditions, detection limits and references

	Spectroscopic characteristics		Experimental conditions: Heavy atom perturber				
Compound	Wavelengths (nm)	Lifetime (μs)	Type	Concentration (M)	$[Na_2SO_3]$ (M)	Detection limit (ng/ml)	Reference
Acenaphthene	290/500	307	$TlNO_3$	0.050	0.0100	65.0[a]	15
	286/493,520	—	KI	—	—	13.9[b]	29
1,5-Dinaphthalensulfonic acid	331/534	464	$TlNO_3$	0.050	0.0100	—	16
1-Hidroxi-2-naftanoic acid	340/510	240	$TlNO_3$	0.050	0.0100	28.2[b]	16
3-Indol butyric (IBA)	288/458	145	KI	1.6	0.0015	24.9[a]	27
α-Naphthylacetic acid	290/495,523	418	$TlNO_3$	0.050	0.0100	109.9[b]	16
(NAA)	292/490,520	236	$TlNO_3$	0.025	0.0015	14.6[a]	27
α-Naphthoxyacetic acid	312/499,525	324	$TlNO_3$	0.050	0.0100	—	16
(α-NOA)	287/495,521	—	KI	—	—	—	26
	297/494,521	—	$TlNO_3$	—	—	—	26
	287/524	350	KI	0.3	0.0100	2.0[b]	30
	297/494,522	340	$TlNO_3$	0.040	0.0100	2.0[b]	30
β-Naphthoxyacetic acid	336/500	307	$TlNO_3$	0.250	0.0100	167.0[a]	15
(β-NOA)	286,323/505,534	332	$TlNO_3$	0.050	0.0100	—	16
	277,320/503,530	670	KI	0.4	0.0100	2.4[b]	30
	284,322/503,529	1230	$TlNO_3$	0.050	0.0100	2.2[b]	30
1-Naphthoxylactic acid (NA)	295/491,527	—	$TlNO_3$	0.065	0.0150	9.6[b]	28
Sodium 1-amino-4-naphthalenosulfonate (1,4-ANS)	—	—	$TlNO_3$	—	—	0.8[b]	22
Sodium 1-amino-5-naphthalenosulfonate	331/534	464	$TlNO_3$	0.050	0.0100	9.3[b]	16
(1,5-ANS)	—	—	$TlNO_3$	—	—	31.3[b]	22
Sodium 1-amino-7-naphthalenosulfonate (1,7-ANS)	—	—	$TlNO_3$	—	—	—	22
Sodium 2-amino-1-naphthalenosulfonate (2,1-ANS)	—	—	$TlNO_3$	—	—	6.3[b]	22
Sodium 2-amino-8-naphthalenosulfonate (2,8-ANS)	—	—	$TlNO_3$	—	—	—	22
α-Bromo naphthalene	299/523,540	446	$TlNO_3$	0.050	0.0100	—	16
(alfa-BrN)	—	—	$TlNO_3$	—	—	—	35
β-Bromonaphthalene (beta-BrN)	275/494,518	—	$TlNO_3$	0.050	0.0100	9.7[b]	21
Carbaryl (CBL)	288/488,526	180	KI	1.4	0.0015	10.6[a]	27
	285/495,523	—	KI	—	—	0.9[b]	41

(Continued on next page)

TABLE 1
Compounds, spectroscopic characteristics, experimental conditions, detection limits and references *(Continued)*

	Spectroscopic characteristics		Experimental conditions: Heavy atom perturber				
Compound	Wavelengths (nm)	Lifetime (μs)	Type	Concentration (M)	$[Na_2SO_3]$ (M)	Detection limit (ng/ml)	Reference
Carbazole (CBZ)	290/440	255	KI	0.6	0.0020	3.8[a]	27
Clorhidrato de 1-naphthylamine	325/545	279	$TlNO_3$	0.050	0.0100	—	16
α-Chloro naphthalene	300/525	398	$TlNO_3$	0.050	0.0100	—	16
	—	—	$TlNO_3$	—	—	—	35
Dansyl chloride	326/574	—	$TlNO_3$	0.050	0.0100	5.1[b]	13
	328/574	541	$TlNO_3$	0.050	0.0100	—	16
Hydroxilated dansyl chloride (DNS-OH)	330/579	—	$TlNO_3$	0.050	0.0100	—	13
Dansylamide (DNSD)	334/620	222	$TlNO_3$	0.200	0.0100	85.7[a]	27
	331/591	778	$TlNO_3$	0.050	0.0100	—	16
Dansyl derivate of aspartic acid (DNS-Asp)	331/590	364	$TlNO_3$	0.050	0.0100	—	13
Dansyl derivate of alanine (DNS-Ala)	330/593	409	$TlNO_3$	0.050	0.0100	—	13
Dansyl derivate of arginine (DNS-Arg)	330/589	60	$TlNO_3$	0.050	0.0100	—	13
Dansyl derivate of asparigine (DNS-Asn)	329/587	500	$TlNO_3$	0.050	0.0100	—	13
Dansyl derivate of phenylalanine (DNS-Phe)	330/590	435	$TlNO_3$	0.050	0.0100	—	13
Dansyl derivate of glutamine (DNS-Gln)	330/590	532	$TlNO_3$	0.050	0.0100	—	13
Dansyl derivate of histidine (DNS-His)	331/594	455	$TlNO_3$	0.050	0.0100	—	13
Dansyl derivate of leucine (DNS-Leu)	332/593	489	$TlNO_3$	0.050	0.0100	—	13
Dansyl derivate of lysine (DNS-Lys)	332/596	608	$TlNO_3$	0.050	0.0100	—	13
Dansyl derivate of metionine (DNS-Met)	333/596	548	$TlNO_3$	0.050	0.0100	—	13
Dansyl derivate of serine (DNS-Ser)	330/593	383	$TlNO_3$	0.050	0.0100	—	13
Dansyl derivate of threonine (DNS-Thr)	330/588	470	$TlNO_3$	0.050	0.0100	—	13
Dansyl derivate of trypthophan (DNS-Trp)	329/584	263	$TlNO_3$	0.050	0.0100	—	13
Dansyl derivate of valine (DNS-Val)	333/591	498	$TlNO_3$	0.050	0.0100	—	13
Phenantrene (PHE)	292/477	—	$TlNO_3$	0.050	0.0100	—	16

(Continued on next page)

TABLE 1
Compounds, spectroscopic characteristics, experimental conditions, detection limits and references *(Continued)*

	Spectroscopic characteristics		Experimental conditions: Heavy atom perturber				
Compound	Wavelengths (nm)	Lifetime (μs)	Type	Concentration (M)	$[Na_2SO_3]$ (M)	Detection limit (ng/ml)	Reference
Phenanthrine	283/482,504	—	KI	—	—	57.2[a]	33
Fluorene (FLU)	295/450	130	$TlNO_3$	0.050	0.0015	14.6[a]	27
	285/467	—	KI	—	—	14.3[b]	29
9-Hydroxy-4-metoxyacridine (HMA)	402/584	91	KI	1.0	0.0005	20.6[a]	27
4-Methylpropranolol	296/526	—	$TlNO_3$	0.085	0.050	21.0[b]	40
Nafazoline (NPZ)	288/488	632	KI	1.0	0.0100	44.0[a]	15
Nafronyl (NFL)	292/492,524	156	KI	1.6	0.0020	11.7[a]	27
Naphthalene (NAPH)	296/482,512	—	$TlNO_3$	0.050	0.0100	—	16
α-Naphthaleneacetamide (NAD)	292/492,540	251	KI	1.2	0.0015	7.8[b]	23
	292/492,540	251	KI	1.2	0.0015	7.8[b]	27
Sodium β-naphthalenesulphonate	284/500,528	223	$TlNO_3$	0.050	0.0100	—	16
1-Naphthylamine	326/546	352	$TlNO_3$	0.050	0.0100	—	16
1-Naphthylamine diacetic acid (NADA)	321/571	—	$TlNO_3$	—	—	—	38
Naphtopidil	296/494,526	475	$TlNO_3$	0.085	0.0085	21.0[b]	32
	282/525	—	KI	1.4	0.005	7.9[b]	36
	—	—	KI	1.4	0.0075		39
β-Naphtol ethyl eter	324/507	408	$TlNO_3$	0.050	0.0100	—	16
Naproxene (NAP)	334/510,540	1028	$TlNO_3$	0.200	0.0040	17.6[a]	27
Pyrene	334/600	—	$TlNO_3$	0.050	0.0100	—	16
	330/596	1330	KI	2.0	0.0100	—	31
	330/596	2230	$TlNO_3$	0.095	0.0100	4.0[b]	31
Propranolol	294/492,526	1290	KI	3.6	0.005	14.4[a]	43
	288/494,522	—	KI	—	—	—	37
	294/492	—	KI	—	—	14.4[a]	42
Thiabendazole (TBZ)	300/488	89	KI	0.8	0.0010	15.4[a]	27
Tryptamine (TRA)	284/452	90	KI	0.8	0.0030	19.7[a]	27
Trypthophan (TRP)	288/446	166	KI	1.0	0.0020	11.2[a]	27

[a]Methodology based in the calibration (44).
[b]IUPAC methodology (45).

phosphorescence (NP-RTP). To find out more about this new phenomenon they studied the substituent group effects and the chemical structure of compounds favouring NP-RTP emissions. Their investigations showed that NP-RTP is not an exceptional phenomenon peculiar to certain derivatives but a normal characteristic of naphthalene derivatives. The basic structure of naphthalene derivatives is their rigid naphthalene nucleus and planar molecular configuration, leading to high fluorescence efficiency. If the conjugated system grows with the substituent group, the electron is easily excited and both phosphorescence and fluorescence are engendered. Thus, electron-donating substituent groups, particularly those with an *n* electron, tend to increase phosphorescence intensity, since they increase the mobility of the electrons in the naphthalene ring. Substituent groups with a negative charge can enhance phosphorescence intensity because in the aqueous solution the proximity between the luminophor and the inorganic heavy-atom perturber, Tl^+, may be increased due to the attraction created by static electricity and may thus enhance the efficiency of intersystem crossing to the triplet state. In heavy-atom substituted compounds the phosphorescence signal

was relatively strong. Finally, compounds with substituted –NO or –OH groups do not favor the emission of phosphorescence in solution (see Table 1).

Nomenclature

This new technique was developed simultaneously by two different research groups, one from Tsing Hua University and our own from Granada University. The former group have chosen to name it non-protected, room-temperature phosphorescence (NP-RTP) (13), while Segura Carretero et al. has called it heavy-atom-induced, room-temperature phosphorescence (HAI-RTP) (15). The term "non-protected, room-temperature phosphorescence" takes this new method to be a simplification of older phosphorescence methods in solution, based on the use of organized (protected) media. Thus our research group prefer the term "heavy-atom-induced, room-temperature phosphorescence" because it describes more accurately the fact that the phosphorescence is induced by the presence of a heavy-atom perturber in the absence of oxygen. In fact, it not possible to observe a phosphorescent signal in the absence of a heavy atom, even when using sodium sulphite as deoxygenator.

Whatever the name, this technique represents a great innovation in obtaining phosphorescent signals and can be included in the general scheme of classification as an alternative to other methods of obtaining and analysing phosphorescence emission in solution. Nevertheless, the fact that the signal is obtained with no kind of organized medium and without any restriction to the kind of analyte involved leads us to suggest that a revised classification of techniques in solution should distinguish between those which require organised media (MS-RTP and CD-RTP) and those which do not (Sensitized and HAI-RTP) (see Figure 1).

Theoretical Justification

The development of this new technique is based first on certain physical-chemical aspects of phosphorescence emission in the presence of sodium sulphite and relatively high concentrations of heavy-atom perturber, and secondly on instrumental development in recent years.

The rate of intersystem crossing can be enhanced by the presence of heavy atoms. Since the probability of the intersystem crossing process includes spin-orbital matrix elements, which depend upon the atomic number of the atoms in the vicinity of the excited electron, phosphorescence emission increases with halogen-substituted aromatics concomitantly with the atomic weight of the halogen. This so-called "internal heavy-atom effect" is also known to quench fluorescence and decrease the lifetime of phosphorescence (17). In 1952, Kasha (18) demonstrated the possibility of stimulating luminescence by the use of an external heavy atom. This effect is called the "external heavy atom effect" and in this case the atoms do not chemically unite the phosphorescent compound.

The use of relatively high concentrations of a heavy-atom perturber permits sufficient interaction between the perturber and the analyte to produce an effective population of its triplet state and thus intense phosphorescence (Figure 2). The intensity of the interaction between the magnetic moment of an electron and the nucleus depends on the distance between them. In micellar media this distance depends on the longitude of the micellar agent chain, which is in the order of 8 to17 Å depending on the micellar agent used, a distance that this new method minimizes.

In this method the medium's lack of viscosity (because no organized medium is used) increases the bimolecular deactivation constant and therefore causes a concomitant decrease in phosphorescence quantum efficiency. Quantum efficiency is inversely proportional to the concentration of the quencher, that is, oxygen. Due to its relatively long lifetime the triple state is generally prone to many deactivation processes. Nevertheless, this drawback is counteracted by the better spin-orbital coupling due to the closer proximity of the heavy-atom perturber to the

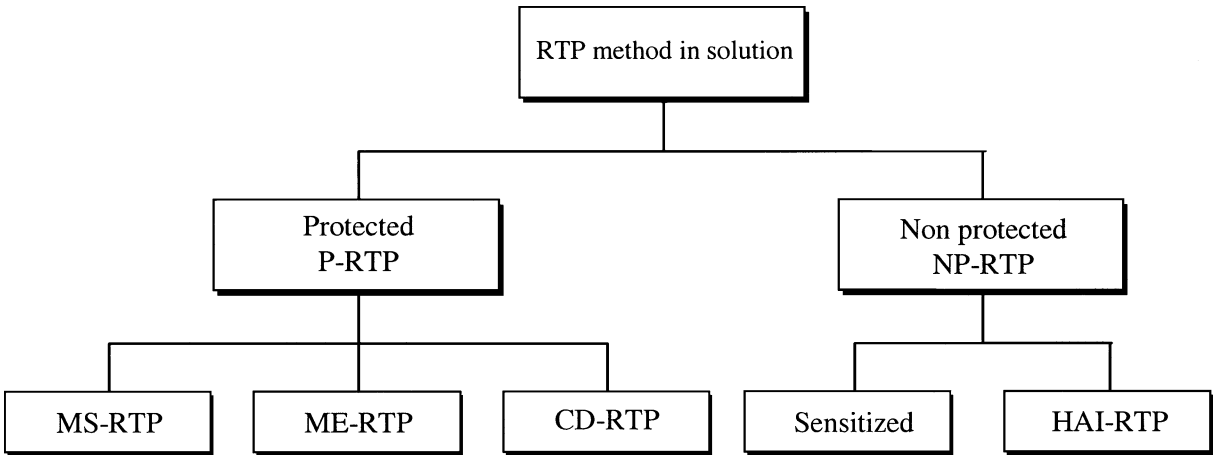

FIG. 1. Classification of RTP method in solution.

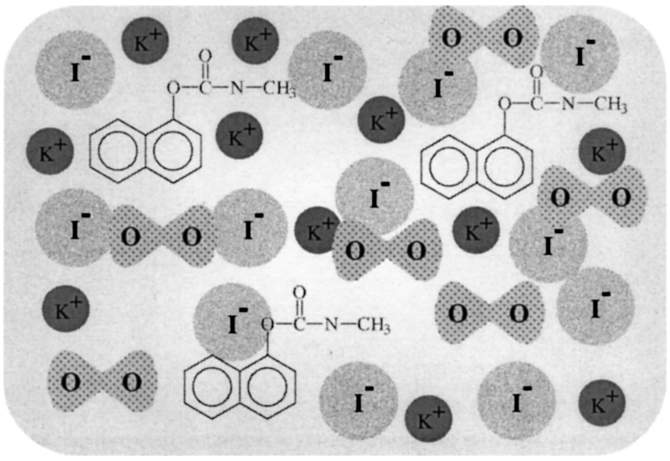

FIG. 2. Schematic representation for justification emission of heavy-atom-induced, room-temperature phosphorescence.

aromatic structures of the phosphors, and so quantum efficiency increases.

The invention of the pulse lamp and the role of modern electronics in molecular luminescence in the last 40 years have permitted the development of new more sensitive instrumentation controlled by microprocessors and multidimensional detectors (19, 20).

Experimental Progress

In this part, the experimental progress of the method is described. The different analytes studied have been reported in Table 1.

Mou et al. (21) demonstrated that a strong, stable, RTP signal from ß-bromonaphthalene (ß-BrN) in an aqueous solution with a peak wavelength of λ_{ex} 275 nm can only be induced by using Na_2SO_3 as an oxygen scavenger and irradiating the solution for some time with the light source of the apparatus. RTP intensity and the irradiation time required to acquire a stable RTP signal are very sensitive to the kind and amount of organic solvent added to the system. For a ß-BrN/Na_2SO_3 aqueous solution system containing 2.5% acetonitrile, a short irradiation time is needed and a strong, stable RTP signal is triggered quickly. RTP intensity was linear to the ß-BrN concentration in the range of 8.0×10^{-8} to 1.6×10^{-5} M with a detection limit of 4.7×10^{-8} M.

The same authors studied the NP-RTP properties of different substituent positions of naphthylmine sulfonate (22). A strong, stable NP-RTP signal from 1-naphthylamine-4-sulfonate (1,4-ANS), 1-naphthylamine-5-sulfonate (1,5-ANS) or 2-naphtylamine-1-sulfonate (2,1-ANS) in aqueous solution can only be obtained by using Na_2SO_3 as deoxygenator and $TlNO_3$ as heavy-atom perturber, and irradiating the solution for a short time with the light source of the apparatus. The detection limits with 1,4-ANS, 1,5-ANS, 2, 1-ANS were 3.8×10^{-9}, 1.4 10^{-7} and 2.8×10^{-8} mol/l respectively. Under the same conditions 1-naphthylamine-7-sulfonate and 1-naphthylamine-8-sulfonate did not emit any RTP signal.

A simple, quick, phosphorimetric method to determine residues of a plant-growth regulator, α-naphthaleneacetamide, in commercial samples was proposed by Cruces Blanco et al. (23). They investigated the effects of different heavy-atom salts (KI, KBr, NaBr, NaI, RbCl, CsCl, KCl, $Cr(NO_3)_3$, $CeCl_3$, $InCl_3$, $Ca(NO_3)_2$, $La(NO_3)_3$, $TlNO_3$, $Pb(NO_3)_2$, and $AgNO_3$) and sodiumsulphite concentrations upon phosphorescence signals. The resulting HAI-RTP spectra were successfully applied to the measurement of α-naphthaleneacetamide concentrations in the range of 7.8 to 100.0 ng/ml, with relative standard deviations of between 2.6 percent and 5.7 percent.

Until now the research group at Tsing Hua University has only used $TlNO_3$ as heavy-atom perturber (12, 13, 21, 22), but other heavy-atom salts can also be used to induce intersystem crossing (15, 23–25). These authors indicated that a strong, stable RTP signal can be elicited from alpha-naphthalenyloxyacetic acid (alpha-NOA) in an aqueous solution by using Na_2SO_3 as deoxygenator and KI (or $TlNO_3$) as heavy-atom perturber (26). The maximum phosphorescence-intensity wavelengths were found to be $\lambda_{ex}/\lambda_{em} = 287/495{,}521$ nm with KI (or $TlNO_3$) as heavy-atom perturber. Although the RTP signal is lower with KI as heavy-atom perturber than it is with $TlNO_3$ the analytical characteristics of the former method are better. The kind and amount of organic solvent added to the system has a decided effect upon RTP intensity, the irradiating time required to acquire a stable RTP signal and the choice of heavy-atom perturber.

Until now no reports on this phenomenon in compounds other than naphthalene derivatives have been published. Thus, to determine the validity of this technique and to see whether other analytes display this characteristic, the authors of this review studied the RTP emission properties of various other polycyclic, aromatic hydrocarbons such as naphthalene derivatives (naphthylacetic acid, α-naphthalene acetamide, naproxen, nafronyl, dansylamide, and carbaryl), fluorene and several nitrogen heterocyclic compounds, such as benzimidazole derivatives (thiabendazole), carbazole, indole derivatives (tryptamine, tryptophan and indole-3-butyric acid), and an acridine derivative (9-hydroxy-4-methoxyacridine) (27). The experimental results demonstrate that it is not only naphthalene derivatives that generate RTP without a protective ordered medium but also nitrogen heterocyclic compounds. In this work we show the value of applying this new RTP method to analysing different kinds of compounds and to understanding the principles of HAI-RTP emission in order to apply it to other different fields such as environmental, clinical or pharmaceutical chemistry. We detail the phosphorescence spectral characteristics of these compounds (excitation and emission wavelengths and lifetime) and the optimization of the chemical variables involved in the phosphorescence phenomenon in solution. Calibration graphs and the detection limit at the ng/mL level have been established under optimum experimental conditions.

In 2000, a new Spanish research group from the University of Castilla La Mancha proposed a method to determine 1-naphthoxylactic acid (28) by obtaining a phosphorescence signal from this analyte ($\lambda_{exc/em}$ 295/491, 527 nm) with no protective medium, using $TlNO_3$(6.5×10^{-2} M) as heavy-atom perturber and Na_2SO_3(1.5×10^{-2} M) as deoxygenator (pH 7.5). Optimization of the operational conditions resulted in a detection limit for 1-naphthoxylactic acid of 9.6 ng/ml according to the error propagation theory.

To improve the applicability of this methodology, Li et al. (29) engaged in different studies into polycyclic aromatic hydrocarbons. These authors reported that it is possible to obtain an intense, stable phosphorescent signal from fluorene and acenaphthene in an aqueous solution containing one percent acetonitrile using only Na_2SO_3 as oxygen scavenger and KI as heavy-atom perturber. The detection limits calculated by IUPAC based on three times the standard deviation of the background were 8.6×10^{-8} and 9.0×10^{-8} M for fluorene and acenaphthene, respectively. The relative standard deviations (n = 7) were 2.4 percent and 3.5 percent for the systems containing 1.2×10^{-5} M of fluorene or acenaphthene.

These authors also studied the NP-RTP properties of α-naphtyhyloxyacetic acid (α-NOA) and β-naphthyloxyacetic acid (β-NOA) and the effects of organic solvents to clarify the effects of the position of the substituent upon luminescence (30). Aqueous solutions of both acids emitted strong, stable RTP signals in the absence of any protecting medium upon the addition of KI or $TlNO_3$ as heavy-atom perturber and Na_2SO_3 as deoxygenator. The fluid RTP systems were completely transparent and stable. Satisfactory linearity between concentration and RTP intensity was obtained with detection limits of 1.0×10^{-8} and 1.2×10^{-8} M for α- and β-naphthytlyloxyacetic acid, respectively. The kind and quantity of organic solvent added to the luminescent system not only affected RTP intensity and the preirradiation time required to attain a stable RTP signal but also the selection of heavy-atom perturber. Under the same conditions the RTP intensity of the system using $TlNO_3$ was much stronger than that of the system using KI, but the detection limits of both systems for analytical determination were comparable. The RTP intensity of β-NOA was lower than that of α-NOA but the influence of the organic solvent on its RTP emission was also less.

These authors also studied the phosphorescence emission of pyrene in solution and found that the delayed excimer fluorescence signal located at 475 nm could be induced in the absence of any protective medium using only KI or $TlNO_3$ as heavy-atom perturber and Na_2SO_3 as deoxygenator (31). The optimum conditions and effects of the kind and quantity of heavy-atom perturber and organic solvents used upon the luminescence properties of the pyrene solution (0.01 M of Na_2SO_3 and 0.095 M of $TlNO_3$) were studied and a detection limit of 2×10^{-8} M was obtained.

Murillo et al. reported on the direct determination of naftopidil (4-2-methoxyphenyl)-α-[1-naphthalenyloxy)methyl]-1-piperazineethanol) via this method (32), by which they obtained a phosphorescence signal using $TlNO_3$ (0.085 M) as heavy-atom perturber and Na_2SO_3 (0.05 M) as deoxygenator. Under these conditions naftopidil emits phosphorescence at an emission wavelength of 526 nm and an excitation wavelength of 296 nm in the range of 0.05 to 1.00 mg/l. Optimization of the various conditions led to their obtaining a detection limit of 21.0 ng/ml according to the error propagation theory.

The phosphorescent characteristics of phenanthrine have been researched by Chen et al. (33). These authors described a strong, stable RTP signal from phenanthrine in an aqueous solution (482 and 504 nm, exciting at 283 nm) in the absence of any protecting medium, using Na_2SO_3 and KI, and established a method with a detection limit of 2.6×10^{-8} M.

Although the phosphorescent characteristics of naphthalene derivatives have already been established (16), the kinetic parameters of their phosphorescent emission using this new method have not so far been reported. The influence of the concentration of heavy-atom perturber on the RTP lifetime of several naphthalene derivatives has been studied and two different ways set out of proving that the phothopysical parameters for RTP emission can be determined on the basis of changes in RTP lifetimes (34).

1-Chloronaphthalene and 1-bromonaphthalene were chosen as the modal compounds and the possibility of determining the photophysical parameters for the emission of NP-RTP according to RTP lifetime method was studied based on the definition of phosphorescence lifetime and its relationship with the concentration of PAH (35). The results obtained in two different ways prove that the RT-lifetime method can be used to determine the photophysical parameters for RTP emission.

NP-RTP has been applied by Murillo et al. (36) to the determination of naftopidil in biological fluids. Optimum conditions were found to be 1.4 M of KI, 0.005 M Na_2SO_3 and pH 6.5. This method has been successfully applied to the analysis of naftopidil in human serum and urine with recoveries of 104.0 ± 0.6 percent and 106.0 ± 1.0 percent, respectively.

A direct and simple RTP to determine propranolol was proposed by Long et al., who used KI as heavy-atom perturber and sodium sulphite as deoxygenator. They looked into the effect of the concentration of KI upon RTP lifetime and calculated the luminescence kinetic parameters. The method was applied directly to urine and drug tablets and the recoveries were between 96.6 percent and 97.4 percent, with a relative standard deviation of 2 percent for 1.0×10^{-6} to 4.0×10^{-6} mol/l propranolol in a spiked urine sample (37).

Chen et al. (38) studied the properties of fluorescence and NP-RTP and the cation recognition of 1-naphthylamine diacetic acid (NADA) with Ca^{2+}, Mg^{2+}, Zn^{2+}, Cd^{2+}, and Tl^{+}. The maximun phosphorescence intensity wavelengths are $\lambda_{exc/em}$ = 321/571 nm, using Na_2SO_3 as deoxidisation and $TlNO_3$ as heavy atom perturber.

A modified simplex method (MSM) has been applied to the determination of dependent variables affecting phosphorescence. We determined naftopidil in urine and serum

(39). The best conditions obtained were 1.4 M of KI, 7.5×10^{-3} of Na_2SO_3 and 5.0×10^{-2} M of Na_2HPO_4/NaH_2PO_4 buffer solution. The recoveries were 103.8 ± 0.8 percent in urine and 99.5 ± 0.5 percent in serum, which are better results than those obtained by using HAI-RTP followed by the classical optimisation. This research group studied the appropriate experimental conditions for the determination of 4-methylpropranolol in cerebrospinal fluid, serum and urine (40). The detection limit, according to the error propagation theory, was 6.2 ng/mL. The optimum concentration of KI was 3.2 M, Na_2SO_3 7.0 10^{-3} M and the accurate value pH 10.9.

A strong and stable signal RTP ($\lambda_{exc/em} = 285/495{,}523$ nm) can be induced for carbaryl (CBL) aqueous solution in the absence of any protective medium only using KI as a heavy atom perturber and sodium sulphite as a deoxygenator (41). The method presents a detection limit of 0.9 ng/ml. The methods was applied satisfactorily to spiked water and soil samples. The influence of KI concentration on RTP lifetime of CBL was studied in detail.

Segura Carretero et al. (42) recently published the direct determination of propranolol by HAI-RTP. The phosphorescence intensity was measured at 492 nm, exciting at 294 nm. The method presents a linear concentration range of between 0 and 500 ng/ml with a detection limit of 14.4 ng/ml and an analytical sensitivity of 6.7 ng/ml. The method was applied satisfactorily to a commercial product, with a recovery percentage of 98.8 and an RSD of 0.6 percent. The method was also validated with a standard addition method of calibration.

Other compounds also have been studied for a thesis in our research group (43), and recently new research has been published (46–55).

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