An introduction to the use of

Fluorescence Spectroscopy in Inorganic Analysis

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Introduction

Fluorescence spectroscopy is widely used in the qualitative and quantitative analysis of inorganic compounds. Phosphorescence spectroscopy is used infrequently, except in the area of inorganic phosphors. Although several inorganic substances are fluorescent or phosphorescent in the solid state, the majority of analyses occur in solution.

Either direct or indirect methods are used in the fluorimetric analysis of inorganics. In the direct method, the native fluorescence of the analyte is used, for example, in the analysis of the uranyl ion. In the indirect method, the analyte is converted to a fluorophore by complexation with an organic compound, for example, in the determination of selenium, diaminonaphthalene is used as a label.

The majority of the methods are steady state measurements. However, it has been shown that kinetic methods may offer certain advantages.

For a more detailed description and specific applications of the use of fluorescence spectroscopy in inorganic analysis the reader is referred to the reviews in Analytical Chemistry and to the references.

Inorganic phosphors

The yellow-green emission of the uranyl ion UO_2^{2+} is well known and the emission of hexavalent uranium dispersed in sodium fluoride by melting a mixture of the two at 1100 °C forms the basis of a very sensitive analysis [1]. All uranyl salts in aqueous solution show a similar emission, which consists of a series of lines centered around 510 nm. The electronic transitions producing the spectrum are partly forbidden and the luminescence has a mean lifetime of 1 msec [2].

The efficiency and lifetime of the uranyl emission depends very much upon the state and concentration of the anion and the solvent. For example, in 1 M H_3PO_4, a lifetime of 187 µsec has been recorded, in 1 M H_2SO_4, 10.8 µsec and in 0.1 M $HClO_4$, 2.1 µsec. The background fluorescence from the solvents and impurities can be reduced using time-based discrimination of the uranyl emission. When combined with solvent extraction of the uranyl ion, a specific and sensitive assay results [70].

In addition to uranyl ions, thallous ions show an emission in sodium chloride solution as do cerium and tin in sulphuric acid. Lead [67] and bismuth [60] can be determined by measuring the emission of the oxide-base obtained after calcining the coprecipitate of the metal with calcium oxalate.

Inorganic luminescence is usually associated with metal ions (dopants, activators) embedded in a relatively inert matrix (host) such as a metallic oxide, phosphate, silicate, etc. The luminescence and lifetimes of these phosphors are determined by the concentration of these ions or activators in the matrix [92]. Great care has to be taken in preparation of these phosphors as minute traces of certain ions such as iron can substantially reduce the intensity. Zinc sulphide containing copper as activator has a green phosphorescence. Other matrices include cadmium, calcium and strontium sulphides, silicates of zinc and calcium, and oxides of yttrium and cadmium. Activators include copper, silver, manganese, thallium, rare earths and uranium. Europium-activated phosphors are used as the red-emitting component of color television tubes. Another well known activator is divalent manganese added to calcium halophosphates and used as fluorescent lamp phosphors. The luminescence of ruby is due to chromium-activated aluminium oxide $AI_2O_3(Cr^{3+})$.

The common materials used in CRT phosphors are listed in Table 1.

The rare earth luminescence spectra are relatively complex and the emission takes place from more than one energy level (3).
The mean lifetime of the different transitions from Eu^{3+}. Y_2O_3 show an increase with increasing emission wavelength (Figure 1).
Detailed examination of the various transitions can provide much information regarding the chemical environment of the activating ion, its position in the crystal lattice, etc. For example, if the rare earth occupies the center of symmetry of the crystal, only magnetic

dipole transitions are possible. For the $^5D_0$ excited state of Eu^{3+} ion, the only transition possible is the $^5D_0$-$^7F_1$, centered around 595 nm, appearing orange as in Eu^{3+} in $NaLuO_2$. If the Eu^{3+} ion does not occupy a center of symmetry, then electron-dipole transitions are additionally possible such as $^5D_0$-$^7F_2$, $^7F_4$, etc. For $^5D_0$-$^7F_2$, the emission is centered around 611 nm appearing red as in Eu^{3+} in $NaGdO_2$.

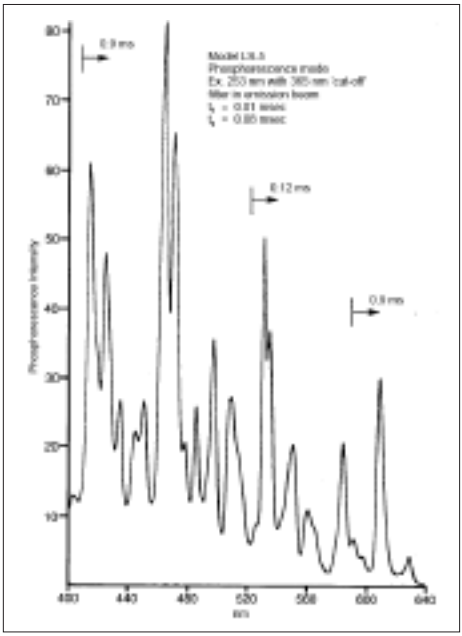


Figure 1. Phosphorescence spectrum of Eu/Y_2O_3 0.54 mol%.

A technique known as SEPIL – selective excitation of probe ion luminescence – has been used by several workers [24, 68, 77] in the analysis of various lanthanides. The method requires the formation of a crystalline host lattice containing a lanthanide ion whose transitions are sharp and modified by the lattice. A tunable dye laser is used to excite a specific ion at a specific site. Er^{3+} [24] has been estimated by coprecipitation with calcium fluoride. Non-fluorescent ions such as La, Ce, Gd, Lu, have been determined by coprecipitating them with Er^{3+} and calcium fluoride. After ignition of the precipitate at high temperature and the cooling to 13 K, the association of Er^{3+} with the non-fluorescent ion is excited with the dye laser. Detection limits of around 4 pg have been observed. Further detailed information is beyond the scope of this introduction and the reader is referred to read the many excellent and interesting books which are devoted to the subject of the physics and chemistry of rare earths and phosphor manufacture [4].

Table 1. Common Materials used in CRT Phosphors.

Host	Dopants (Percentage of dopant atoms)		Persistence (s)
ZnS	Ag, Cl	10^{-2}	10^{-5}
Zn-CdS	Cu, Al	10^{-2}	10^{-1}
CaS	Ce		10^{-7}
Y_2O_2S	Eu	1	10^{-7}
Gd_2O_2S	Tb	1	10^{-3}
Zn_2SiO_4	Mn	1	10^{-2}
	Mn	1	
	As	10^{-2}	1
$Zn_3(PO_4)_2$	Mn	1	10^{-2}
$KMgF_3$	Mn	2	1

Organo-metallic complexes

As mentioned earlier, the vast majority of luminescence inorganic analysis is carried out by complexing the metal ion with a fluorescent, or non-fluorescent organic molecule. The addition of the metal ion to a molecule containing several fused ring systems confers rigidity which favors the development of fluorescence. Mention should be made at this point of the difference between the photochemistry of transition or rare earth metals bound to chelating reagents and those of non-transition elements. In the case of the latter, the metal ion forms a part of the overall ring system and the fluorescence is derived from a lowering of the $\pi^* - \pi$ energy level relative to the $\pi^* - n$ state of the chelating groups.

In the former, intramolecular energy transfer occurs from the excited energy levels of the ligand to the 4f levels of the lanthanide ions [73]. The emission occurs at similar wavelengths to the free lanthanide ion but with a much higher intensity [5].

Rare Earth Complexes

The combination of rare earth ions and a ligand forms the basis of a highly sensitive assay for the rare earth ions and also of a means of probing biological macromolecules such as proteins. The β-diketonate complexes of europium are highly fluorescent and Yamada [6] has described a very sensitive assay with detection limits of 2 pg L^{-1} using tris (1,1,1-trifluoro-4-[thionyl]-2-4 butanedione), a nitrogen laser and pulse-gated photon counting. Maximum absorption is at 339 nm with the emission occurring at several bands centered around 614 nm. The emission lifetime of complex was found to be 4.2 msec. Residual fluorescence from organic impurities, Raman signals from the solvent and scattered light were removed by gating the detection to give a 3 µsec delay after the end of excitation. The following table lists some typical assays for various rare earth elements. Lanthanides, in particular Tb^{3+}, have been used as fluorescent probes in biological molecules for many years [7]. The free Tb^{3+} ion in aqueous solution has negligible fluorescence, but on binding to certain protein molecules such as apoferritin, a 105 increase in intensity is observed, together with an increase in the mean lifetime.

The spectra of the free Tb^{3+} ion and also its complex with transferrin are shown in Figure 2. The lifetimes of the two species are shown in Figure 3. The protein residues involved in the energy transfer have been identified as being those of tryptophan. The excitation spectrum of the bound terbium differs significantly from that of the free form and closely resembles that of the UV absorption of tryptophan residues.

The energy transfer mechanism probably involves transfer of triplet state energy from the amino acid to the terbium. Displacement of bound Tb^{3+} by other cations competing for the same site provides an insight into the number and types of bonding sites. Other rare earths used as probes include europium for monitoring the excited electronic states and energy transfer characteristics of nucleotides, for example the Eu^{3+}-RNA complex. Intramolecular energy transfer has also been observed in ytterbium-porphyrin complexes.

Diamagnetic Metal Ion Complexes

The formation of a fluorescent chelate by the combination of a diamagnetic metal ion and an organic ligand proves to be a sensitive and specific method for many metal ions, particularly those which are difficult to measure by atomic absorption spectroscopy. The basic requirement of the ligand is the ability to bond covalently with the metal ion through at least two functional groups to form a rigid ring structure. The metal ion acts as a Lewis acid (electron pair acceptor) while the ligand acts as a Lewis base (electron pair donator). Typical of these functional groups are -COOH, -OH, $-NH_2$ and the free ligand may be weakly fluorescent or not at all.

Upon complexation, the fluorescence properties of the ligand are enhanced and show a batho-chromic shift. There are exceptions: the Zr-flavanol chelate emission is blue, while flavanol fluorescence is green. Some ligands are specific for certain metals and facilitate selective fluorescence analysis of these ions.

Table 2. Typical Ion Fluorimetric Assays and Reagents.

Rare Earth ion	Method of analysis	Ligand	Limit of Detection ppb	Ex	Em	Ref.
Erbium	SEPIL	CaF_2	0.025	446	550	24
Europium	OMC	Benzoyltrifluoroacetone	3	320	615	25
	OMC	2-theonyltrifluoroacetone	1	390	615	26
	OMC	111 trifluoro-2-thionyl 2-4 butanedione	2	339	614	6
	OMC	Hexafluoroacetone trioctylphosphineoxide	1	360	615	24
Samarium	OMC	2-theonyltrifluoroacetone	0.1	400	560	26
Terbium	OMC	Hexafluoroacetone trioctylphosphineoxide	100	350	550	24
Metal ion	**Chelating Reagent**		**Limit of Detection ppM**	**Ex**	**Em**	**Ref.**
Aluminium	Acid alizarin garnet red		0.007	470	580	40
	Superchrome blue		0.02	330	640	41
	8-Hydroxyquinoline		0.10	365	520	42
	Lumogallion		0.001	465	555	30
			0.00006	470	555	31
Boron	Quinalizarin		0.01	365	595	45
	Benzoin		0.05	410	480	35
	Carminic acid		0.1	476	556	36
Beryllium	Morin		0.01	420	525	34
Calcium	Calcein		0.2	410	490	44
Gallium	Rhodamine β		0.01	450	650	45
	2(2-pyridilbenzimidazole)		0.07	347	413	46
	Lumogallion		0.001	490	570	47
Lead	4 NH_2 Ph-EDTA		0.08	360	450	48
Magnesium	8-Hydroxyquinoline		0.01	420	530	49
	8-Hydroxyquinoline 5 sulphonic acid		0.01	374	505	49
Manganese	Carminic acid		0.09	467	556	51
Selenium	3,3-Diaminobenzidine		0.02	425	565	75
	2,3-Diaminonaphthalene		0.02	374	525	39
Tin	8-Hydroxyquinoline 5 sulphonic acid		0.005	360	515	52
	3,4,7-Trihydroxyflavone		0.001	427	473	55
	3-Hydroxyflavone		0.004	415	495	74
Zinc	Dibenzothiazolymlmethane		0.5	365	450	53
	8-Quinolinol		0.5	375	517	54

In analyzing mixtures, separation by solvent extraction is usually required at some specific pH and with a particular solvent. Proper choice of the excitation and emission wavelength can also play a part in increasing the selectivity of analysis. Work has shown that the use of micelles can greatly enhance the fluorescence emission. For example, the niobium-quinol complex fluoresces strongly in the presence of cetrimide.

The excitation/emission spectra are shown in Figure 4.

Probably the first use of metal chelates was in the analysis of aluminium using the pentahydroxyl-flavone called morin. Since then many ligands have been investigated: among them the most common are derivatives of 8-quinolinol, flavanols, benzoin, rhodamines, β-diketones and amino naphthalenes. The structures of some typical ligands are shown below.

Although many assays have been published for a wide variety of metal ions with relatively good sensitivity, nearly all suffer from interference. Table 2 lists some typical fluorimetric assays and reagents for a large number of metal ions.

Both the stoichiometry of the complex, in other words the mole ratio of metal ion to chelating agent, and the stability/dissociation constant can be determined by fluorescence measurement, as illustrated by Brittain [27] who investigated the adduct formation between europium III β-diketonates and various substrates.

There are two general methods in use. The first is the mole-rate method [28], in which fluorescence is measured for a series of solutions containing varying concentrations of either ligand or metal ion while the other is kept constant. The number of metal ions bound corresponds to where the curve changes slope. Where there is a more gradual change, the stoichiometry is given by the intersection of two tangents; one through the origin and tangential to the first part of the curve, and one drawn tangential to the later, horizontal part of the curve. The second is Job's method of continuous variation [29]. Here, the fluorescence intensity is measured for a series of solutions each containing different ratios of metal ion to ligand but with the same total concentration. The maximum number of metal ions bound corresponds to that solution which fluoresces maximally.

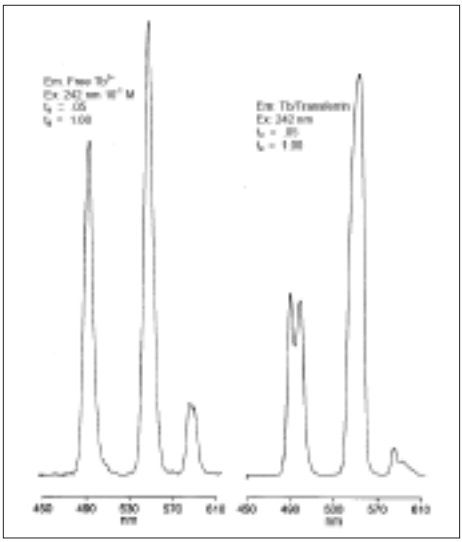


Figure 2. Terbium-transferrin spectra as measured with a Model LS-55.

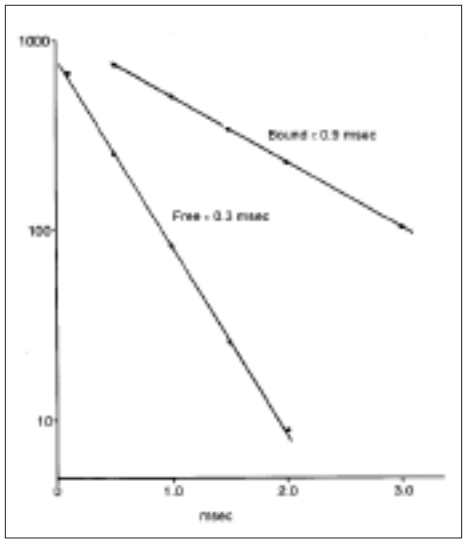


Figure 3. Terbium lifetimes as measured with a Model LS-55.

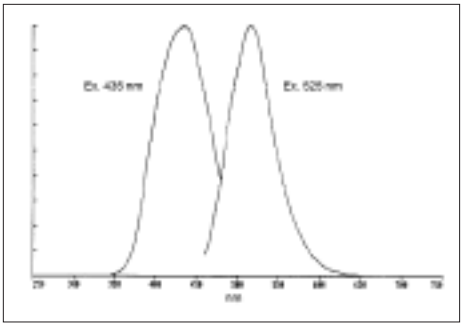


Figure 4. Fluorescence excitation/emission of niobium-quinol in cetrimide micelles.

The most useful fluorescence assays in terms of simplicity, relative freedom of interference from competing ions and better sensitivity than atomic absorption spectrometry, are those for aluminium, beryllium, boron and selenium. Only these methods will be discussed in detail as it is beyond the scope of this review to provide a comprehensive discussion as to the specific reagent, pH, choice of solvent excitation and emission wavelengths and interferences for all metal ions. Additional information is provided in the above table and in the References section of this booklet.

Aluminium

Aluminium forms chelates with a wide variety of chelating agents, for example, acid alizarin red, solochrome dark blue, morin, and 8-hydroxyquinoline. Lumogallium has been reported as offering excellent sensitivity and freedom from interference. A particularly useful analysis is the determination of aluminium in sea water [30, 31] where, although atomic absorption has the required sensitivity [32], severe interference results from the excessively high salt concentration. In the reaction, a solution containing aluminium is heated with a buffered solution of lumogallium at 80 ˚C for 20 minutes and the complex formed measured at an excitation of 465 nm and an emission of 555 nm. The sensitivity of the assay enables concentrations of less than 1 µg L^{-1} to be determined. Using a laser and time discrimination to reduce the background counts from Rayleigh and Raman

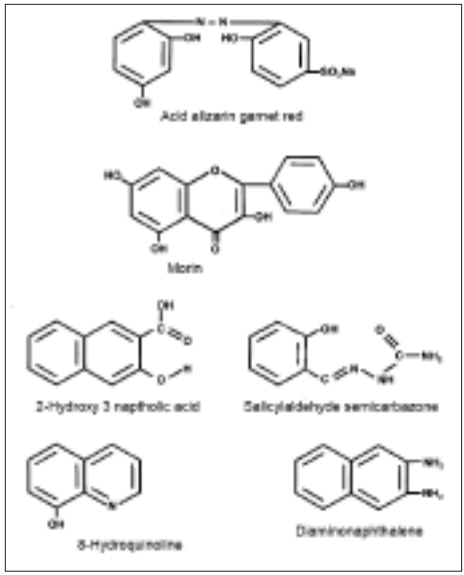


Figure 5. Typical cationic chelating agents.

scatter, Haugen et al [33] found a calculated detection limit of 0.06 µg L^{-1} for Al^{3+}. However, this could only be achieved for extra high purity solvents and reagents.

Beryllium

Beryllium can be determined in river water at concentrations around the 0.2 µg L^{-1} level using a modified Sill and Willis [34] method. The assay is based upon the fluorescence of the beryllium-morin complex and interferences from iron and rare earths are eliminated by complexing with triethylamine (TEA) and diethyltriamine pentaacetate (DTPA). Perchloric acid is used to solubilize the beryllium and the pH adjusted until the blue fluorescence of quinine is extinguished. A piperidine buffer and the morin are then added and after 20 minutes, the fluorescence is measured with an excitation of 420 nm and an emission of 525 nm. The fluorescence is linear for 0-10 µg in 50 ml sample.

Boron

Boron is a biologically important element and is found as a natural constituent of soil, plants

and water. Irrigation water normally contains 0.01 to 0.3 ppm, but occasionally up to 5 ppm. Since it is used in a number of products (soap, detergents, fertilizers, steels and cements), it is a potential environmental contaminant. A number of analytical procedures have been published; among them is (a) complexing of the borate ion with benzoin [35] and (b) with carminic acid [36]. Alkaline solutions of benzoin are easily oxidized and, in addition, dissolved oxygen has a quenching effect. The fluorescence intensity at Ex 410 nm and Em 480 nm is also time-dependent and varies with the type of solvent. However, when measured in a formamide buffer with isopropylamine, there is a linear relationship between intensity and concentration between 0.05 and 0.5 µg ml^{-1} boron. The reaction with carminic acid in a buffered neutral solution gives a maximum fluorescence emission at 556 nm when excited at 407 nm. Disodium EDTA is added to prevent calcium phosphate precipitation and, apart from magnesium, no other serious interferences are encountered.

Selenium

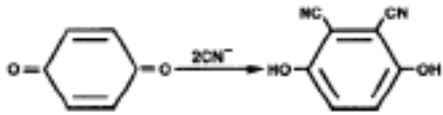
Many papers have been published on the analysis of selenium, particularly in biological material, for example urine [83], and also in potable waters and sea water [81]. The assays are predominantly based upon the fluorescence of the selenium 2,3-diaminonaphthalene (DAN) complex formed by reacting selenium, as the selenite, with DAN and then extracting the complex with cyclohexane. The assay is sensitive enough to be carried out in water containing less than 0.01 µg ml^{-1} of selenium and, under certain assay conditions, little interference is observed from either inorganic or organic contaminants [21]. Linear calibration up to 5 µg ml^{-1} is obtained at an excitation wavelength of 374 nm and an emission at 525 nm. When analyzing biological material, care should be taken to prevent loss of selenium through volatilization, particularly when halogens are present. Wet digestion using nitric acid or perchloric acid in Kjeldhal flasks is a generally accepted method giving good recoveries for selenium.

Anions

Anion assays may be classified into five groups according to the type of reaction involved. These are: (a) redox reaction, (b) complex formation, (c) ion association, (d) enzyme reaction, and (e) substitution. For a more comprehensive review, the reader is referred to an extensive review on anion assay by Gomez-Hens and Valcarel [61].

Cyanide

Cyanide ions reacts with p-benzoquinone to form a highly fluorescent derivative with the cyanide ion acting as a reducing agent. The proposed reaction is giving a sensitivity range of 0.2-50 µg ml^{-1} at an excitation of 400 nm emission 480 nm. Anions such as sulphide and thiocyanate do not interfere with the reaction. Various other substituted quinines have been investigated together with various solvents [62].



Fluoride

Although many of the assays published for fluoride are based on substitution reaction, the ternary complex found with zirconium and calcium blue provides the only assay which is relatively free from interference. The method is rapid and has sensitivity in the ppb range. The reaction requires a pH of 2.5 and the excitation is at 350 nm with an emission of 410 nm.

Phosphate

An ion association complex between molybdophosphate and rhodamine B provides the basis for an assay for phosphorous at 0.04 to 0.6 µg. The complex is extracted into chloroform butanol (4:1) and the fluorescence measured at 575 nm with an excitation at 350 nm, after first extracting excess of the rhodamine with chloroform. Large amounts of silicate and arsenic do not interfere and the complex

has been shown to have a ratio of 3 moles rhodamine B to 1 mole molybdophosphate [64].

Phosphate can also be assayed using an enzyme reaction with glycogen phosphorylase [65]. The latter converts glycogen into glucose-1-phosphate which is converted to glucose-6-phosphate with phosphoglutomutase.

A third reaction in which the fluorescent $NADH_2$ is found from glucose-6-phosphate and NADP is used as the marker. Enzymatic cycling has been used to measure sensitivity to 10^{-13} mole inorganic phosphate. However, care has to be taken regarding purity of the solvents, buffers and enzymes to avoid large blank values.

Conclusion

Though atomic absorption spectroscopy overwhelms the literature in the area of quantitative inorganic analysis, fluorescence spectroscopy fulfills an important need, particularly for those elements such as aluminium, boron and selenium. In the area of anionic analysis, fluorescence spectroscopy can provide the basis for a highly specific and sensitive assay, for example, phosphate ion by enzymic cycling.

In general, the identification of metal ions in mixtures by comparing spectra of the chelates is not possible due to the lack of identifying features in their emission spectra. Liquid chromatography has been used in an attempt to separate various ions. A derivative of EDTA was used to chelate Zn, Cd and Pb and following chromatography fluorescamine was used to give a fluorescent derivative [80].

Improved detection limits have been observed for metal ions such as Co [84], Cr [85], Mn [87] and V [89] by using chemiluminescence measurements. Anions such as nitrate and nitrite [89-91] have also been measured. Another interesting area of research is in the use of micelles to improve the detection limit of some fluorescing metalchelate complexes. With the advent of new techniques and instrumentation, fluorescence and other forms of luminescence spectroscopy will continue to be used successfully in inorganic analysis.

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