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FLUORIMETRY: A SIMPLE, RAPID AND SENSITIVE ANALYTICAL TECHNIQUE- A REVIEW

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Abstract: Fluorimetry is a type of rapid and sensitive electromagnetic spectroscopy that analyzes fluorescence from a sample by using a beam of light, usually UV light that excites the electrons in molecules of certain compounds and causes them to emit light. Emission of light can be affected by concentration, pH, solvent, and structure of molecule. Compared to other investigative techniques, fluorimetry has a high specificity, simplicity, and low cost. It is well recognized as a strong approach utilized in a range of fields including environmental research, industrial research, medical diagnostics, forensic investigation, genetic analysis, and biotechnology and chemistry.

Index Terms **- Fluorimetry, simplicity, analysis.**

I. INTRODUCTION

Fluorimetry is an analytic method for detecting and measuring fluorescence in compounds that uses ultraviolet light stimulating the compounds, causing them to emit visible light. The energy/light emitted by the substance has a linger wavelength than absorbed. This process of emitting radiation with a longer wavelength than absorbed is known as luminescence (cold light) [1].

1.1 Fluorescence

Fluorescence is a type of luminescence caused by photons exciting a molecule, raising it to an electronic excited state. It is an optical phenomenon in which the molecular absorption of energy in the form of photons triggers the emission of fluorescent photons with a longer wavelength.

1.1.1 The Mechanism of Fluorescence

Fluorochromes will only fluoresce if they are illuminated with light of the corresponding wavelength. The wavelength depends on the absorption spectrum of the fluorophore and it has to be ensured that an appropriate quantity of energy is delivered to elevate the electrons to the excited state. After the electrons are excited they can dwell in this high energy state for a very short time only. When the electrons relax to their ground state or another state with a lower energy level, energy is released as a photon. As some of the energy is lost during this process, light with an increased wavelength and lower energy is emitted by the fluorochrome compared to the absorbed light. F spectroscopy provides two types of spectrum (1) Excitation or absorption spectrum & (2) Emission spectrum.

1.2 Phosphorescence

Phosphorescence is a specific type of photoluminescence related to fluorescence. Unlike fluorescence, a phosphorescent material does not immediately re-emit the radiation it absorbs. The slower time scales of the re-emission are associated with "forbidden" energy state transitions in quantum mechanics. As these transitions occur very slowly in certain materials, absorbed radiation may be re-emitted at a lower intensity for up to several hours after the original excitation [3].

1.3 The Mechanism of Phosphorescence

As phosphorescing molecules can luminesce for a much longer time than fluorochromes, there must be a difference in the way they store the excitation energy. The basis for this discrepancy is found in the two forms of excitation levels, the singlet excited state and the triplet excited state, which are based on different spin alignments [1,2].

II. ELECTRONIC STATES IN FLUORIMETRY

Understanding the difference between fluorescence and phosphorescence requires the knowledge of electron spin and the differences between singlet and triplet states.

According to the Pauli Exclusion Principle, two electrons in an atom cannot have the same four quantum numbers {Principal (n), Azimuthal (ℓ), Magnetic (m_ℓ), and Spin quantum number (s)}. Only two electrons can occupy each orbital where they must have opposite spin states. These opposite spin states are called spin pairing. Because of this spin pairing, most molecules do not exhibit a magnetic field and are diamagnetic.

In diamagnetic molecules, electrons are not attracted or repelled by the static electric field. Free radicals are paramagnetic because they contain unpaired electrons that have magnetic moments that are attracted to the magnetic field [3].

2.1 Singlet State

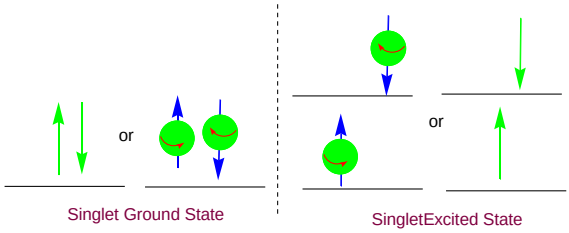
When all the electron spins are paired in the molecular electronic state and the electronic energy levels do not split when the molecule is exposed to UV radiation.

If there is n number of unpaired electrons, it means that (n+1) fold degeneracy (equal energy state) will be associated with the electron spin, regardless of the molecular orbital occupied. Thus if no unpaired electrons are present (n=0),

According to the formula: n+1,

0+1 = 1 Spin state (singlet state)

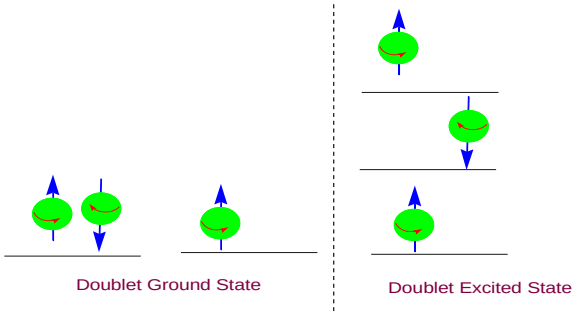
Figure-1 Singlet state



2.2 Doublet State

A doublet state occurs when there is an unpaired electron that gives two possible orientations when exposed to UV radiation and imparts different energy to the system.

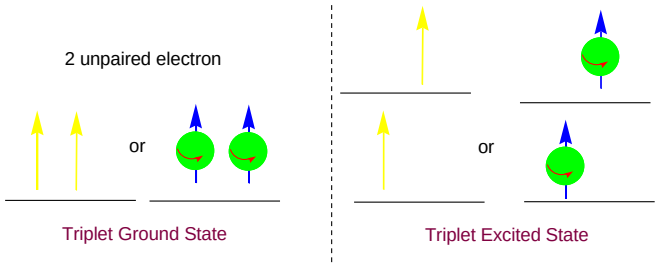
Figure-2 Doublet state



A singlet or a triplet can form when one electron is excited to a higher energy level. In an excited singlet state, the electron is promoted in the same spin orientation as it was in the ground state (paired).

In a triplet, excited stated, the electron that is promoted as the same spin orientation (parallel) to the other unpaired electron.

Figure-3 Triplet state



Singlet, doublet, and triplet is derived using the equation for multiplicity,

$$2S+1,$$

Where:-

S is the total spin angular momentum (sum of all the electron spins).

Individual spins are denoted as spin up (S = +1/2) or spin down (S= -1/2).

If we were to calculate the S for the excited singlet state, the equation would be 2(+1/2 + -1/2) +1 = 2(0) +1 = 1, therefore making the center orbital in the figure a singlet state.

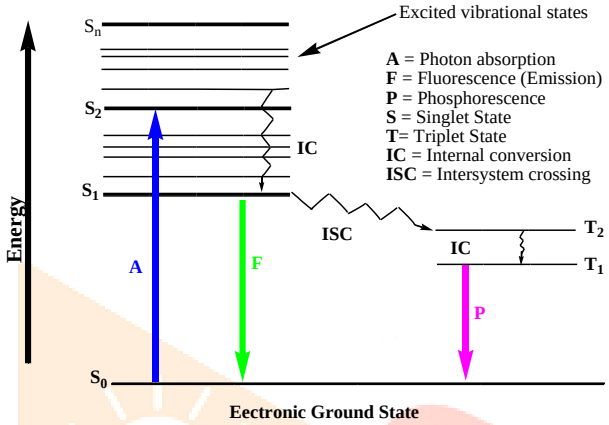
If the spin multiplicity for the excited triplet state was calculated, we obtain 2(+1/2 + +1/2)+1 = 2(1)+1 =3, which gives a triplet state as expected [2].

The difference between a molecule in the ground and the excited state is that the electrons are diamagnetic in the ground state and paramagnetic in the triplet state. This difference in the spin state makes the transition from singlet to a triplet (or triplet to singlet) more improbable than the singlet-to-singlet transitions. This singlet to triplet (or reverse) transition involves a change in the electronic state. Due to that, the lifetime of the triplet state is longer the singlet state by approximately 10 seconds fold difference.

The radiation that induced the transition from ground to excited triplet state has a low probability of occurring, thus their absorption bands are less intense than singlet-singlet state absorption. The excited triplet state can be populated from the excited singlet state of certain molecules which results in phosphorescence [1,3].

These spin multiplicities in the ground and excited states can be used to explain the transition in photoluminescence molecules by the Jablonski diagram.

Figure- 4 Jablonski diagram



Once a molecule has absorbed energy in the form of electromagnetic radiation (longer wavelength, that is upward-pointing, blue arrow, S_0 ---> S_1, S_2, ...S_n), there are a number of routes by which it can return to ground state. If the photon emission (short wavelength that is downward-pointing, green arrow) occurs between states of the same spin state (S1 ---> S0) it is called fluorescence. If the spin state of the initial and final energy levels are different (T1 --> S0), the emission (loss of energy) is called phosphorescence. In the diagram, this is depicted by a longer wavelength (lower energy) and therefore shorter length pink line. Since fluorescence is statistically much more likely than phosphorescence for most molecules, the lifetimes of fluorescent states are very short and phosphorescence somewhat longer. Three non-radiative deactivation processes are also significant here: internal conversion (IC), intersystem crossing (ISC), and vibrational relaxation [4].

2.3 Internal Conversion

It is an intermolecular process by which a molecule passes to a lower energy electronic state without emission of light. Overlap of vibrational energy levels in two electronic energy levels.

2.4 External conversion

External conversion is a process in which excited molecules lose their energy due to collisions with other molecules or by transfer of their energy to solvent or other unexcited molecules. Therefore, the external conversion is influenced by temperature, solvent viscosity, as well as solvent composition.

2.5 Intersystem crossing

In this process spin of an excited electron is reversed and change in multiplicity results. Most common when vibrational manifold overlap exists and when the molecule has a heavy atom substituent (e.g. Br, I) [2,4].

III. FACTOR AFFECTING FLUORESCENCE

3.1 Effect of Structural Nature

The nature of the chemical structure of a molecule in terms of flexibility and rigidity is of major influence on the fluorescence and phosphorescence signal. Molecules that have a high degree of flexibility will tend to decrease fluorescence due to higher collisional probability. However, more rigid structures have a lower probability of collisions and thus have more fluorescence potential. For example, Biphenyl has very low fluorescence quantum efficiency due to the flexible nature of the molecule while fluorine has high fluorescence quantum efficiency due to its rigidity.

1,1'-biphenyl — **Flexible**

9H-fluorene — **Rigid**

3.2 Effect of Solvent Nature

Solvents affect the luminescent behavior of molecules. There are three common effects can be recognized –

(1) The polarity of Solvent - A polar solvent is preferred as the energy required for the P ---> P* is lowered.

(2) The viscosity of Solvent- Highly viscous solvent is preferred since collisional deactivation will be lowered at higher viscosities.

(3) Heavy Atoms in Solvent - If solvents contain heavy atoms, fluorescence quantum efficiency will decrease and phosphorescence will increase.

3.3 Effect of Substitution

Substitution in the structure can also affect the fluorescence

Functional groups increase the fluorescence intensity	OH, OMe, OEt, CN, NHR, NH2, NR2, NO, NO2
Functional groups decrease fluorescence intensity	COOH, CHO, COR, COOR, SH, F, Cl, Br, I
Functional groups having no effect on fluorescence intensity	SO3 H, NH4 +, Alkyl groups

3.4 Effect of Temperature

Molecule experiences larger collisional deactivation at high temperatures due to an increase in the movement and velocity of molecules. Therefore, lower temperatures are preferred for analysis.

3.5 Effect of Dissolved Oxygen

Dissolved oxygen affects fluorescence at large scale. Molecules experience intersystem crossing due to it is paramagnetic nature.
Effect of Concentration: - The fluorescence is directly proportional to the amount of absorbed radiation. When the concentration of the fluorescent molecules increases in a sample solution, the fluorescence intensity is reduced [4,5].

3.6 Quenching

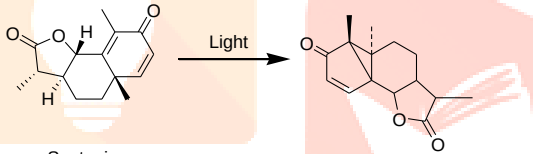
A process that decreases the fluorescence intensity of a sample. A variety of molecular interactions can result in quenching. Like – molecular rearrangement, Static quenching, and collisional quenching, etc.

3.6.1 Excited-State Reactions Quenching

Such reactions occur because light absorption frequently changes the electron distribution within a fluorophore, which in turn changes its chemical or physical properties. For example, a neutral solution of phenol can lose the phenolic proton in the excited state.

3.6.2 Molecular Rearrangement Quenching

It involves the migration of a group or an atom from one center (migration origin) to another (migration terminus) due to light and heat within the same molecule. For example lumisantonin a photoproduct of santonin obtained via molecular rearrangement. The C-3 carbonyl group has moved to C-2, the C-4 methyl has moved to C-1, and the C-10 carbon has been inverted.

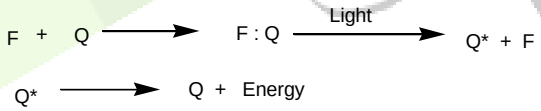


3.6.3 Collisional Quenching

Collisional quenching occurs when the excited fluorophore experiences contact with an atom or molecule that can facilitate non-radiative transitions to the ground state. Common quenchers include O2, I-, Cs+, and acrylamide. For example, quenching of quinine drug by chloride ion and quenching of tryptophan by iodide ion.

3.6.4 Static Quenching

Static quenching occurs at the ground state of the fluorescent molecule. It can be simplified by the following mechanism-



Here, a complex formation occurs between the fluorescing molecule at the ground state (F) and the quencher molecule (Q) through a strong coupling. Such complex may not undergo excitation or, may be excited to a little extent reducing the fluorescence intensity of the molecule. For example, Caffeine and related xanthines and purines reduce the intensity of riboflavin by the static mechanism.

3.7 Concentration

Concentration quenching is a kind of self-quenching. It occurs when the concentration of the fluorescing molecule increases in a sample solution. The fluorescence intensity is reduced in a highly concentrated solution (>50 µg/ml).

3.8 Chemical Quenching

Chemical quenching is due to various factors like change in pH, presence of oxygen, halides, and electron-withdrawing groups, heavy metals, etc.

3.9 Change in pH

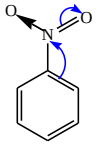
Aniline at pH (5-13) gives fluorescence when excited at 290 nm. But pH <5 or, pH >13 does not show any fluorescence.

3.10 Oxygen Molecules

Oxygen leads to the oxidation of fluorescent substance to non-fluorescent substance and thus, causes quenching.

3.11 Halides and Electron-Withdrawing Groups

Halides like chloride ions, iodide ions, and electron-withdrawing groups like -NO, -COOH, -CHO groups lead to quenching.



Electron withdrawing process by *nitro* groups

3.12 Heavy Metals

The presence of heavy metals also leads to quenching because of collision and complex formation [6].

IV. FLUORESCENT DYES [7]

Table- 1 Fluorescent dyes and their excitation and emission wavelength

Sample Fluorescent Dyes	Excitation	Emission
Indo-1, Ca saturated	331 nm	404 nm
Indo-1 Ca2+	346 nm	404 nm
Cascade Blue BSA pH 7.0	401 nm	419 nm
Cascade Blue	398 nm	420 nm
LysoTracker Blue	373 nm	421 nm
Alexa 405	401 nm	421 nm
LysoSensor Blue pH 5.0	374 nm	424 nm
LysoSensor Blue	374 nm	424 nm
DyLight 405	399 nm	434 nm
DyLight 350	332 nm	435 nm
BFP (Blue Fluorescent Protein)	380 nm	439 nm
Alexa 350	343 nm	441 nm
7-Amino-4-methylcoumarin pH 7.0	346 nm	442 nm
Amino Coumarin	345 nm	442 nm
AMCA conjugate	347 nm	444 nm
Coumarin	360 nm	447 nm
7-Hydroxy-4-methylcoumarin	360 nm	447 nm
7-Hydroxy-4-methylcoumarin pH 9.0	361 nm	448 nm
6,8-Difluoro-7-hydroxy-4-methylcoumarin pH 9.0	358 nm	450 nm
Hoechst 33342	352 nm	455 nm
Pacific Blue	404 nm	455 nm
Hoechst 33258	352 nm	455 nm
Hoechst 33258-DNA	352 nm	455 nm
Pacific Blue antibody conjugate pH 8.0	404 nm	455 nm
PO-PRO-1	434 nm	457 nm

PO-PRO-1-DNA	435 nm	457 nm
POPO-1	433 nm	457 nm
POPO-1-DNA	433 nm	458 nm
DAPI-DNA	359 nm	461 nm
DAPI	358 nm	463 nm
Marina Blue	362 nm	464 nm
SYTOX Blue-DNA	445 nm	470 nm
CFP (Cyan Fluorescent Protein)	434 nm	474 nm
eCFP (Enhanced Cyan Fluorescent Protein)	437 nm	476 nm
1-Anilinonaphthalene-8-sulfonic acid (1,8-ANS)	375 nm	479 nm
Indo-1, Ca free	346 nm	479 nm
1,8-ANS (1-Anilinonaphthalene-8-sulfonic acid)	375 nm	480 nm
BO-PRO-1-DNA	462 nm	482 nm
BOPRO-1	462 nm	482 nm
BOBO-1-DNA	461 nm	484 nm
SYTO 45-DNA	451 nm	486 nm
evoglow-Pp1	448 nm	495 nm
evoglow-Bs1	448 nm	496 nm
evoglow-Bs2	448 nm	496 nm
Auramine O	431 nm	501 nm
DiO	487 nm	501 nm
LysoSensor Green pH 5.0	447 nm	502 nm
Cy 2	489 nm	503 nm
LysoSensor Green	447 nm	504 nm
Fura-2, high Ca	336 nm	504 nm
Fura-2 Ca2+sup>	336 nm	505 nm
SYTO 13-DNA	488 nm	506 nm
YO-PRO-1-DNA	491 nm	507 nm
YOYO-1-DNA	491 nm	509 nm
eGFP (Enhanced Green Fluorescent Protein)	488 nm	509 nm
LysoTracker Green	503 nm	509 nm
GFP (S65T)	489 nm	509 nm
BODIPY FL, MeOH	502 nm	511 nm

Sapphire	396 nm	511 nm
BODIPY FL conjugate	503 nm	512 nm
MitoTracker Green	490 nm	512 nm
MitoTracker Green FM, MeOH	490 nm	512 nm
Fluorescein 0.1 M NaOH	493 nm	513 nm
Calcein pH 9.0	494 nm	514 nm
Fluorescein pH 9.0	490 nm	514 nm
Calcein	493 nm	514 nm
Fura-2, no Ca	367 nm	515 nm
Fluo-4	494 nm	516 nm
FDA	495 nm	517 nm
DTAF	495 nm	517 nm
Fluorescein	495 nm	517 nm
Fluorescein antibody conjugate pH 8.0	493 nm	517 nm
CFDA	495 nm	517 nm
FITC	495 nm	517 nm
Alexa Fluor 488 hydrazide-water	493 nm	518 nm
DyLight 488	493 nm	518 nm
5-FAM pH 9.0	492 nm	518 nm
FITC antibody conjugate pH 8.0	495 nm	519 nm
Alexa 488	493 nm	520 nm
Rhodamine 110	497 nm	520 nm
Rhodamine 110 pH 7.0	497 nm	520 nm
Acridine Orange	431 nm	520 nm
Alexa Fluor 488 antibody conjugate pH 8.0	499 nm	520 nm
BCECF pH 5.5	485 nm	521 nm
PicoGreendsDNA quantitation reagent	502 nm	522 nm
SYBR Green I	498 nm	522 nm
Rhodaminen Green pH 7.0	497 nm	523 nm
CyQUANT GR-DNA	502 nm	523 nm
NeuroTrace 500/525, green fluorescent Nissl stain-RNA	497 nm	524 nm
DansylCadaverine	335 nm	524 nm
Rhodol Green antibody conjugate pH 8.0	499 nm	524 nm

Fluoro-Emerald	495 nm	524 nm
Nissl	497 nm	524 nm
Fluorescein dextran pH 8.0	501 nm	524 nm
Rhodamine Green	497 nm	524 nm
5-(and-6)-Carboxy-2', 7'-dichlorofluorescein pH 9.0	504 nm	525 nm
DansylCadaverine, MeOH	335 nm	526 nm
eYFP (Enhanced Yellow Fluorescent Protein)	514 nm	526 nm
Oregon Green 488	498 nm	526 nm
Oregon Green 488 antibody conjugate pH 8.0	498 nm	526 nm
Fluo-3	506 nm	527 nm
BCECF pH 9.0	501 nm	527 nm
SBFI-Na+	336 nm	527 nm
Fluo-3 Ca2+	506 nm	527 nm
Rhodamine 123, MeOH	507 nm	529 nm
FlAsH	509 nm	529 nm
Calcium Green-1 Ca2+	506 nm	529 nm
Magnesium Green	507 nm	530 nm
DM-NERF pH 4.0	493 nm	530 nm
Calcium Green	506 nm	530 nm
Citrine	515 nm	530 nm
LysoSensor Yellow pH 9.0	335 nm	530 nm
TO-PRO-1-DNA	515 nm	531 nm
Magnesium Green Mg2+	507 nm	531 nm
Sodium Green Na+	507 nm	531 nm
TOTO-1-DNA	514 nm	531 nm
Oregon Green 514	512 nm	532 nm
Oregon Green 514 antibody conjugate pH 8.0	513 nm	533 nm
NBD-X	466 nm	534 nm
DM-NERF pH 7.0	509 nm	537 nm
NBD-X, MeOH	467 nm	538 nm
CI-NERF pH 6.0	513 nm	538 nm
Alexa 430	431 nm	540 nm
Alexa Fluor 430 antibody conjugate pH 7.2	431 nm	540 nm

CI-NERF pH 2.5	504 nm	541 nm
Lucifer Yellow, CH	428 nm	542 nm
LysoSensor Yellow pH 3.0	389 nm	542 nm
6-TET, SE pH 9.0	521 nm	542 nm
Eosin antibody conjugate pH 8.0	525 nm	546 nm
Eosin	524 nm	546 nm
6-Carboxyrhodamine 6G pH 7.0	526 nm	547 nm
6-Carboxyrhodamine 6G, hydrochloride	525 nm	547 nm
Bodipy R6G SE	528 nm	547 nm
BODIPY R6G, MeOH	528 nm	547 nm
6 JOE	520 nm	548 nm
Cascade Yellow antibody conjugate pH 8.0	399 nm	549 nm
Cascade Yellow	399 nm	549 nm
mBanana	540 nm	553 nm
Alexa Fluor 532 antibody conjugate pH 7.2	528 nm	553 nm
Alexa 532	528 nm	553 nm
Erythrosin-5-isothiocyanate pH 9.0	533 nm	554 nm
6-HEX, SE pH 9.0	534 nm	559 nm
mOrange	548 nm	562 nm
mHoneydew	478 nm	562 nm
Cy 3	549 nm	562 nm
Rhodamine B	543 nm	565 nm
DiI	551 nm	565 nm
5-TAMRA-MeOH	543 nm	567 nm
Alexa 555	553 nm	568 nm
Alexa Fluor 555 antibody conjugate pH 7.2	553 nm	568 nm
DyLight 549	555 nm	569 nm
BODIPY TMR-X, SE	544 nm	570 nm
BODIPY TMR-X, MeOH	544 nm	570 nm
PO-PRO-3-DNA	539 nm	571 nm
PO-PRO-3	539 nm	571 nm
Rhodamine	551 nm	573 nm
Bodipy TMR-X conjugate	544 nm	573 nm

POPO-3	533 nm	573 nm
Alexa 546	562 nm	573 nm
BODIPY TMR-X antibody conjugate pH 7.2	544 nm	573 nm
Calcium Orange Ca2+	549 nm	573 nm
TRITC	550 nm	573 nm
Calcium Orange	549 nm	574 nm
Rhodaminephalloidin pH 7.0	558 nm	575 nm
MitoTracker Orange	551 nm	575 nm
MitoTracker Orange, MeOH	551 nm	575 nm
Phycoerythrin	565 nm	575 nm
Magnesium Orange	550 nm	575 nm
R-Phycoerythrin pH 7.5	565 nm	576 nm
5-TAMRA pH 7.0	553 nm	576 nm
5-TAMRA	549 nm	577 nm
Rhod-2	552 nm	577 nm
FM 1-43	472 nm	578 nm
Rhod-2 Ca2+	553 nm	578 nm
Tetramethylrhodamine antibody conjugate pH 8.0	552 nm	578 nm
FM 1-43 lipid	473 nm	579 nm
LOLO-1-DNA	568 nm	580 nm
dTomato	554 nm	581 nm
DsRed	563 nm	581 nm
Dapoxyl (2-aminoethyl) sulfonamide	372 nm	582 nm
Tetramethylrhodamine dextran pH 7.0	555 nm	582 nm
Fluor-Ruby	554 nm	582 nm
Resorufin	571 nm	584 nm
Resorufin pH 9.0	571 nm	584 nm
mTangerine	568 nm	585 nm
LysoTracker Red	578 nm	589 nm
Lissaminerhodamine	572 nm	590 nm
Cy 3.5	578 nm	591 nm
Rhodamine Red-X antibody conjugate pH 8.0	573 nm	591 nm
Sulforhodamine 101, EtOH	578 nm	593 nm

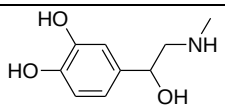
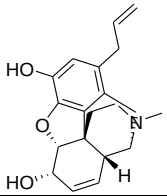
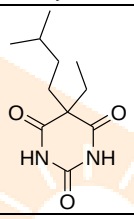
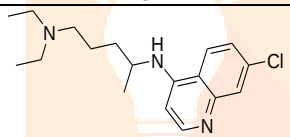
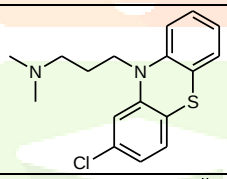
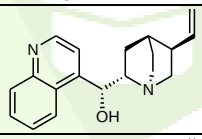
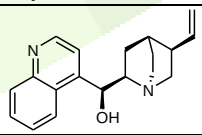
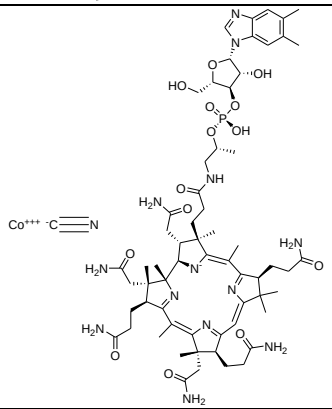
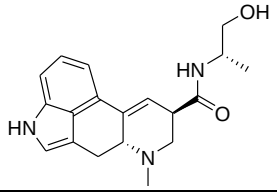
JC-1 pH 8.2	593 nm	595 nm
JC-1	592 nm	595 nm
mStrawberry	575 nm	596 nm
MitoTracker Red	578 nm	599 nm
MitoTracker Red, MeOH	578 nm	599 nm
X-Rhod-1 Ca2+	580 nm	602 nm
Alexa Fluor 568 antibody conjugate pH 7.2	579 nm	603 nm
Alexa 568	576 nm	603 nm
5-ROX pH 7.0	578 nm	604 nm
5-ROX (5-Carboxy-X-rhodamine, triethylammonium salt)	578 nm	604 nm
BO-PRO-3-DNA	574 nm	604 nm
BOPRO-3	574 nm	604 nm
BOBO-3-DNA	570 nm	605 nm
Ethidium Bromide	524 nm	605 nm
ReAsH	597 nm	608 nm
Calcium Crimson	589 nm	608 nm
Calcium Crimson Ca2+	590 nm	608 nm
mRFP	585 nm	608 nm
mCherry	587 nm	610 nm
Texas Red-X antibody conjugate pH 7.2	596 nm	613 nm
HcRed	590 nm	614 nm
DyLight 594	592 nm	616 nm
Ethidium homodimer-1-DNA	528 nm	617 nm
Ethidiumhomodimer	528 nm	617 nm
Propidium Iodide	538 nm	617 nm
SYPRO Ruby	467 nm	618 nm
Propidium Iodide-DNA	538 nm	619 nm
Alexa 594	590 nm	619 nm
BODIPY TR-X, SE	588 nm	621 nm
BODIPY TR-X, MeOH	588 nm	621 nm
BODIPY TR-X phallacidin pH 7.0	590 nm	621 nm
Alexa Fluor 610 R-phycoerythrin streptavidin pH 7.2	567 nm	627 nm
YO-PRO-3-DNA	613 nm	629 nm

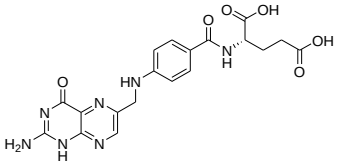
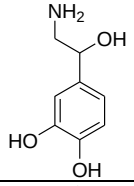
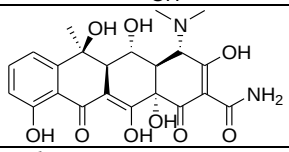
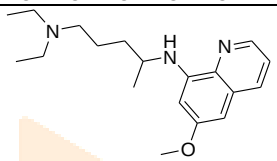
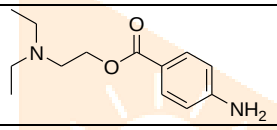
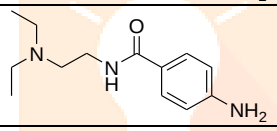
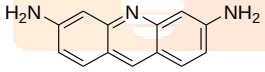
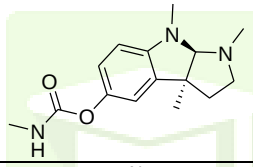
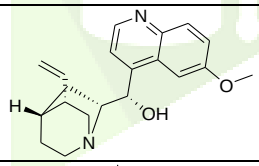
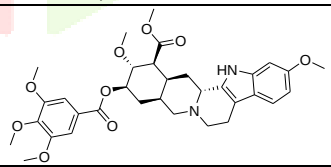
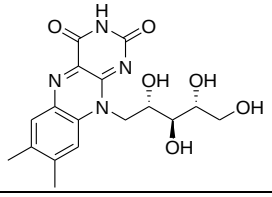
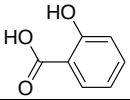
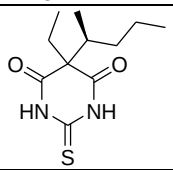
Di-8 ANEPPS	469 nm	630 nm
Di-8-ANEPPS-lipid	469 nm	631 nm
YOYO-3-DNA	612 nm	631 nm
Nile Red-lipid	553 nm	636 nm
Nile Red	559 nm	637 nm
DyLight 633	624 nm	646 nm
mPlum	587 nm	649 nm
TO-PRO-3-DNA	642 nm	657 nm
DDAO pH 9.0	648 nm	657 nm
Fura Red, high Ca	434 nm	659 nm
Allophycocyanin pH 7.5	651 nm	660 nm
APC (allophycocyanin)	650 nm	660 nm
Nile Blue, EtOH	631 nm	660 nm
TOTO-3-DNA	642 nm	661 nm
Cy 5	646 nm	664 nm
BODIPY 650/665-X, MeOH	646 nm	664 nm
Alexa Fluor 647 R-phycoerythrin streptavidin pH 7.2	569 nm	666 nm
DyLight 649	652 nm	668 nm
Alexa Fluor 647 antibody conjugate pH 7.2	653 nm	668 nm
Alexa 647	653 nm	669 nm
Fura Red Ca2+	435 nm	670 nm
Atto 647	644 nm	670 nm
Fura Red, low Ca	472 nm	673 nm
Carboxynaphthofluorescein pH 10.0	600 nm	674 nm
Alexa 660	664 nm	691 nm
Alexa Fluor 660 antibody conjugate pH 7.2	663 nm	691 nm
Cyanine-5.5	673 nm	692 nm
Alexa Fluor 680 antibody conjugate pH 7.2	679 nm	702 nm
Alexa 680	679 nm	703 nm
DyLight 680	678 nm	706 nm
Alexa Fluor 700 antibody conjugate pH 7.2	696 nm	719 nm
Alexa 700	696 nm	720 nm
FM 4-64, 2% CHAPS	506 nm	751 nm

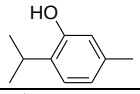
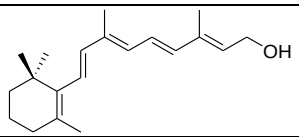
FM 4-64	508 nm	751 nm

V. FLUORESCENT COMPOUNDS [2]

Table- 2 Fluorescent Compounds related pH, wavelength and minimum concentration.

Compound	Structure	pH	Wavelength(nm) Fluorescence	Minimum Concentration
Adrenaline		1	335	0.1
Allyl morphine		1	355	0.1
Amobarbital		14	410	0.1
Chloroquine		11	400	0.05
Chlorpromazine		11	480	0.1
Cinchonidine		1	445	0.01
Cinchonine		1	420	0.01
Cyanocobalamine		7	305	0.003
Ergometrine		1	465	0.01

Folic acid		7	450	0.01
Noradrenaline		1	320	0.006
Oxytetracycline		11	520	0.05
Pamaquine		11	530	0.06
Procaine		11	345	0.01
Procainamide		11	385	0.01
Proflavine		1	510	0.01
Physostigmine		1	360	0.04
Quinine		1	450	0.002
Reserpine		1	375	0.008
Riboflavine		6	520	0.01
Salicylic acid		11	435	0.01
Thiopentone		13	530	0.1

Thymol		7	300	0.1
Vitamin A			470	0.01

VI. APPLICATIONS OF FLUORIMETRY

6.1 Applications in inorganic/ organic chemistry

- Determination of ruthenium
- Determination of aluminum in alloys
- Determination of chromium and manganese in steel
- Determination of uranium salts
- Estimation of rare earth terbium
- Estimation of bismuth
- Determination of beryllium in silicates
- Determination of cadmium
- Assay of thiamine
- Estimation of quinine sulphate
- Estimation of 3,4 benzpyrene

6.2 Investigation of chemical structures and reactions

Applied in the investigation of-

- Hydrogen Bonding
- Cis and Trans isomerism
- Polymerization
- Tautomerism
- Rates of reactions etc.
- Free radicles: The free radicles can best be detected with a spectrograph so that the whole spectrum of a short lived component may be photographed at the same time [8].

6.3 Other Applications

- Quantitative as well as qualitative analysis
- Human cancer diagnosis (Laser induced fluorescence spectroscopy)
- Study of marine petroleum pollutants
- Accurate determination of glucose
- Fluorescence polarization immunoassay of mycotoxins
- Determination of fluorescent drugs in low-dose formulations in the presence of non-fluorescent excipients.
- Determination of impurities where the impurity is fluorescent.
- Study of the drugs complex formulations.
- Widely used in bio-analysis for measuring small amounts of drug and for studying drug-protein binding [8,9].

VII. CONCLUSION

Fluorimetry is a sensitive technique in which trial molecules are excited with a photon source that resulting emission of cold light. The molecule being tested can be affected by concentration, binding, solvent, pH value, structure type, and quenching effect. The chief applications of this technique are determination and study of organic and inorganic compounds, immunoassays, cancer cell diagnosis, Study of pollutants and drugs complex formulations etc. However, there are many factors that can compromise your data and invalidate your results. You should always be aware of possible sample contamination and signal contamination by stray or scattered light. Emission spectrum collection and blank inspection are essential for all experiments.

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