



## Spectrofluorimetry At A Glance: A Review

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### Abstract:

A method to find and examine the fluorescence in the sample is fluorescence spectroscopy, fluorimetry, or spectrofluorimetry. An energy shift from a higher to a lower state that is accompanied by radiation is known as fluorescence. When an electron transitions from an excited state to the electronic ground state and releases its excess energy as a photon, the process is known as luminescence, which is the emission of light by a substance. Fluorescence spectroscopy is a rapid and accurate method for understanding the molecular environment and events in materials. Because of its remarkable sensitivity, high specificity, simplicity of use, and low cost fluorimetry is favored over other analytical techniques. It is a reliable technique that is frequently applied in the field of medical diagnosis for a variety of environmental applications. This review portrays a concise account of the introduction to spectrofluorimetry, its principle, factors affecting and its various applications in organic, inorganic, agricultural, chemical, industrial, pharmaceutical, biosciences, environmental and specific applications are also included in this article.

**Keywords:** Fluorescence, Spectrofluorimetry, Spectroscopy etc.

### Introduction:

When molecules relax from electronically excited states, photon emission processes called fluorescence take place. Fluorophores, which are polyatomic fluorescent molecules, move between electronic and vibrational states throughout these photonic processes. The main component of fluorescence spectroscopy is fluorophores. Molecule's fluorophores are the elements that give them this property. The most common fluorophores are tyrosine, tryptophan, fluorescein, and other molecules with aromatic rings. A substance emitting light without being heated is said to be luminescent, making it a type of cold body radiation. Potential reasons include chemical reactions, electrical energy, subatomic vibrations, and crystal stress.

Two conditions must be met for luminescence:

1. Zero band gap is required for the brilliant material. If there is no band gap in a metal, luminescence is not produced. All potential causes.
2. This substance has to receive energy before luminescence can occur.<sup>[1]</sup>

### **Luminescence(photoluminescence):**

When an electron returns to the ground state from an excited state, it produces luminescence which is the spontaneous emission of light by a substance. It loses the extra photon energy. It might be caused by crystal stress, chemical processes, electrical energy, or subatomic vibrations.

Two types of luminescence

1. Fluorescence

2. Phosphorescence

A specific substance will release visible light or radiation when a light beam strikes it. It is called fluorescence.

Fluorescence begins as soon as the light is absorbed and ends as soon as the incident light is turned off. The use of non-ionizing radiation ensures that the fluorescent chemicals, which are responsible for this phenomenon, have the potential for in-situ, near real-time diagnosis. Certain substances emit light continually long after the incident light has been turned off when light radiation is incident on them. Phosphorescence is the name given to this kind of delayed fluorescence. Phosphorescent compounds are those that emit phosphorescence.

Fluorescence spectroscopy is a rapid and accurate method for describing a sample's molecular environment and process. Fluorimetry is preferred over other analytical methods because of its exceptional sensitivity, high specificity, ease of use, and low cost. It is a well-known and effective approach that is applied in a wide range of biotechnology, forensic, environmental, industrial, and medical diagnostic applications. For both qualitative analyses, it is an important analytical tool.

1. Singlet ground state: one in which a molecule's electrons are entirely coupled
2. Doublet state: an atomic configuration in which an unpaired electron is present, such as in a free radical.
3. The triplet state: is a state in which there are unpaired electrons with the same spin.
4. Singlet excited state: An excited state in which the electrons have opposite spins but are unpaired.
5. Collisional deactivation: occurs when all of the energy is lost owing to collisional deactivation and no radiation is released.<sup>[2]</sup>

## Principle:

When UV or visible light is absorbed, electrons move from their singlet ground state to their singlet excited state. Due to the instability of this state, it emits returns to the environment as UV or visible radiation in solitary ground conditions. When the fluorescence emission happens as decay from the excited singlet electronic states to a permissible vibrational intensity in the electrical ground state. The vibration level structures in the ground and excited electronic states, respectively, are reflected in the fluorescence excitation and emission spectra.<sup>[1]</sup>

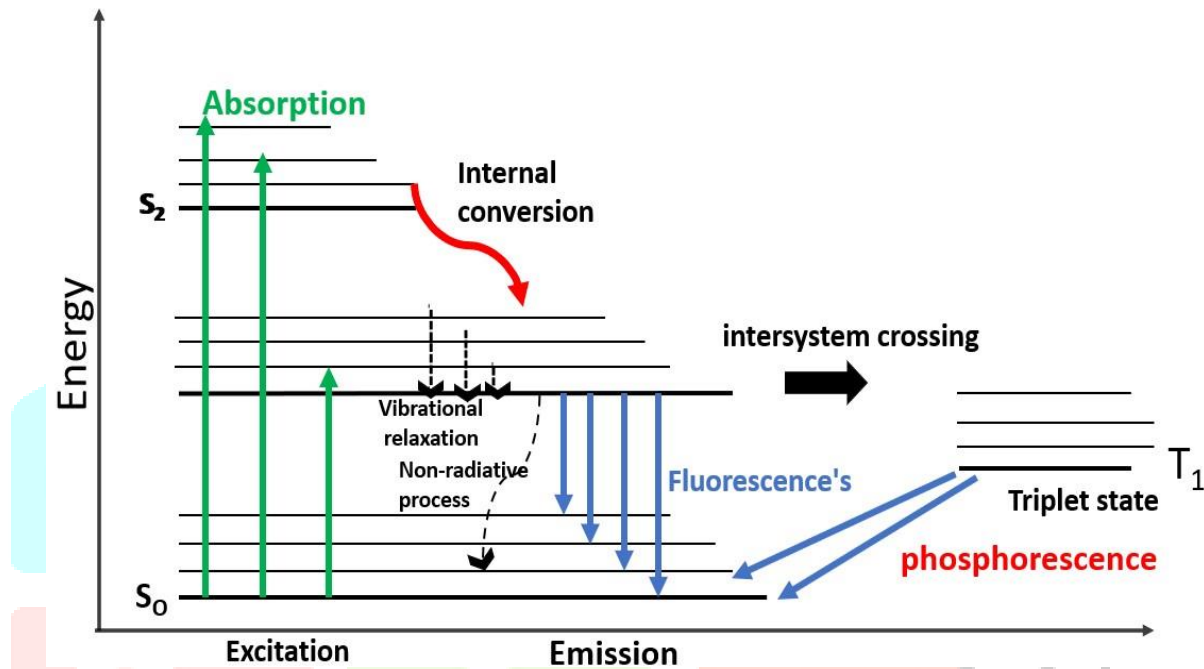


Figure 1: Jablonski Diagram

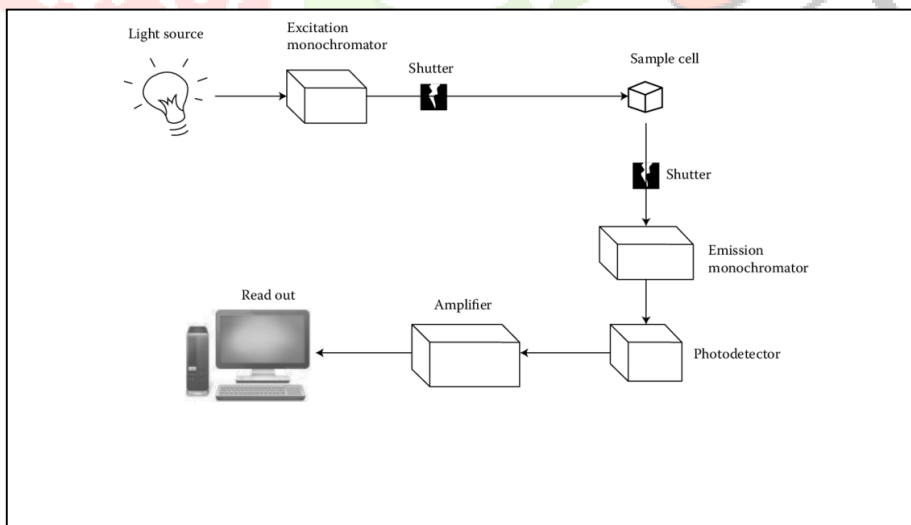


Figure 2: Instrumentation of spectrofluometry

It consists of a continuous ozone-free xenon lamp that produces a continuum of visible and ultraviolet light, a monochromator to choose the necessary wavelength for excitation, a sample compartment, and a second monochromator connected to a photomultiplier tube (PMT) to analyze the fluorescence signal are all components

of the device. The excitation and emission monochromator's grating can scatter light with a wavelength ranging from 200 to 900 nm. Each monochromator's entrance and exit slits regulate the light's intensity and wavelength spread (bandpass). An elliptical mirror collects light from the Xenon lamp and directs it toward the excitation monochromator's entrance slit. A limited range of excitation light wavelengths is selectively delivered to the sample by the excitation monochromator. The sample absorbs a fraction of the incident light and some of the molecules in the sample glow. In order to reduce the possibility of incident light being transmitted or reflected reaching the detector, the emission monochromator is angled at 90 degrees to the path of the excitation light when the emitted light enters it. No monochromator is flawless and it will occasionally transmit stray light, or light with a different wavelength than the intended use. The emission light passes through the adjustable slits within a restricted range that is centered around the designated emission wavelength before impacting the detector. A voltage that is proportionate to the observed emission intensity is produced after the signal is amplified. Front-facing geometry is a type of geometry used for fluorescence collection. In this geometry, the material is irradiated by excitation light at the same surface from which fluorescence is collected. When the sample is opaque or solid, the front face geometry is often used.

By scanning the emission monochromator for a constant wavelength of the excitation light, the fluorescence spectrum of a sample is captured. Similarly, a set emission wavelength is used to scan the excitation monochromator in order to acquire an excitation spectrum. Each wavelength of the source light has a particular intensity, and this intensity might change over time within an experiment or between experiments. To obtain an instrument-independent spectrum, adjustments are required because this may cause the spectrum. The fluorimeter has an additional reference photodiode (PD) that measures a portion of source light that has been divided using a beam splitter (BS) after the excitation monochromator's exit slit but before it enters the sample compartment in order to correct this. Correction for the variation in excitation light intensity as a function of wavelength is achieved by limiting the fluorescence signal to the reference signal.

When measuring molecular fluorescence, the sample is excited at the absorption wavelength, also known as the excitation wavelength, and the emission is observed at a longer wavelength, also known as the emission or fluorescence wavelength. In order to avoid measuring the incident radiation, fluorescence emission is typically recorded at right angles to the incident beam. Fluorescence is the term for the brief emission that takes place, whereas phosphorescence is the term for the considerably longer-lasting luminescence.<sup>[3]</sup>

## Factors:

At low concentrations and within specific boundaries, the intensity of exciting light is proportional to the concentration of the fluorescing substance.

**1.Substituents:** Substitutes have a significant impact on fluorescence. Fluorescence is frequently enhanced by a delocalizing electron substituent, such as the NH<sub>2</sub>, OH, F, OCH<sub>3</sub>, NHCH<sub>3</sub>, and N(CH<sub>3</sub>)<sub>2</sub> groups, because these groups tend to boost the likelihood of a transition between the lowest excited singlet state and the ground state

Fluorescence is reduced or totally quenched by electron withdrawing groups comprising -Cl, Br, -I, NHCOCH<sub>3</sub>, NO<sub>2</sub>, or -COOH

**2. Molecule rigidity:** Molecules with stiff structures are more prone to fluorescence. By reducing vibrations, molecular stiffness diminishes the likelihood of competing non additive transition which reduces intersystem crossover to the triplet state and collisional heat degradation. Fluorescein and eosin, for instance, are intensely fluorescent, whereas phenolphthalein, a chemically related substance that is non-rigid and in which the conjugate system is broken, is not.

**3. Solvent polarity:** Solvent polarity also influences fluorescence and Fluorescence is reduced by solvents with heavy atoms or other similar atoms in their structures.

**4. Dissolved oxygen concentration:** Dissolved oxygen concentration frequently causes the fluorescent substance in a solution to oxidize, diminishes the intensity of the emission. The paramagnetic characteristics of molecular oxygen cause quenching as well.

**5. pH changes:** The fluorescence of substances is also noticeably affected by pH changes. For instance, aniline exhibits a blue fluorescence when stimulated at 290 nm in the pH range of 5 to 13. In extremely alkaline conditions, aniline occurs as the anion and as the aniline cation at lower pH. A cation or an anion cannot glow. Because states have shorter average lifetimes than n states and because fluorescence competition processes are far less likely to occur, fluorescence is more frequently linked with states than states.

**6. Quenching:** Quenching is the reduction in fluorescence intensity caused by a specific reaction between the component of the solution. There are many things that could cause quenching. For instance, excessive primary or fluorescent light absorption by the solution may result in concentration quenching. The inner filter effect is another name for this. The behavior is known as self-quenching if it happens as a result of the fluorescent substance's own absorption.<sup>[4]</sup>

## Applications:

### Inorganic chemistry applications:

#### 1. Determination of ruthenium:

When platinum metal is present, it is determined. Palladium precipitates with the reagent and can be separated by centrifuging. Since iron creates a compound that dims the fluorescence, iron should not be present. Any other platinum group element up to a concentration of at least 30 mL<sup>-1</sup> can be present without affecting the measurement of ruthenium between 0.3 and 2.0 mL<sup>-1</sup>.

## 2. Measuring the boron content of steel:

The Benzoin forms help to determine it. The boron in the sample's acid solution is first converted into boric acid, which is then separated from the remaining substances by co-distillation with methyl alcohol. Boric acid is present in the resultant distillate, which is neutralized by NaOH and evaporated

## 3. Finding the amount of aluminum in alloys:

The reagent is the dye pontochrome blue black which is used in a buffered solution with a PH of 4.8. It is appropriate for steel that contains 0.01 to 1.00% of acid soluble aluminum the basic idea is that an azo dye complex is created when sodium salt of 2, 2-dihydroxy-1,1- azo naphthalene is added to aluminum. The complex fluorescence is measured at 4.9 pH following the electrolysis of mercury to remove aluminum and other impurities.

## 4. Chromium and manganese determination in steel:

Acid is used to dissolve steel, then persulfate is added to the solution to oxidize it. Ions that absorb in the violet and yellow-green, respectively, are  $\text{CrO}_4^{2-}$  and  $\text{MnO}_4^-$ . Although there is some slight overlap in the absorption. Mn can be estimated using a different measurement if a section is treated with  $\text{NaNO}_2$ , which reduces  $\text{MnO}_4^-$  but not  $\text{CrO}_4^{2-}$ . Chromium can be found by measuring the reduced amount at  $\lambda = 4100 \text{ \AA}$ .

## 5. The measurement of uranium salts:

Nitric acid is used to boil the sample before sodium fluoride and uranium fluoride are added to fuse them. The melt cools and solidifies into glass, which can be immediately viewed with a fluorometer that has been made for the purpose. With the reagent, palladium precipitates, which can be separated by centrifuging. Because iron creates a compound that snuffs out the fluorescence, iron should be absent.

## 6. Estimation of rare earth terbium:

Production of a fluorescent complex using EDTA, and salicylic acid. The sulfonosalicylic acid absorption spectra and excitation spectrum are the same. Peaks in the fluorescence spectra may be seen at 4850, 5450 and 6300  $\text{\AA}$ , which are associated with the ion Tb.

## 7. Bismuth estimation:

In order to absorb the radiation, the solutions are first vaporized in an argon hydrogen flame and then exposed to the iodine emission line at 2061.63  $\text{\AA}$ , which is quite close to the bismuth line at 2061.70  $\text{\AA}$ .

## 8. Determination of beryllium in silicates:

Beryllium levels in silicate the creation of a fluorescent beryllium and morin combination. By using mercury cathode electrolysis, interferences like iron and rare earths are eliminated. Triethanolamine and diethylene triamine pentaacetate are then used to complicate the complex's fluorescence.



## 8. Calculating 3,4-Benzpyrene:

This cancer-causing substance is removed by solution from tobacco or tobacco smoke deposits, separated by chromatography using (A1203), and then eluted after that, the fluorescent solution is put in a glass cell and exposed to radiation using a mercury lamp and glass filter.

## 10. Zinc determination:

The following approach is based on the zinc complex of oxime, which fluoresces in ultraviolet light. 5.0, 10.0, 15.0, 20.0, and 25.0 ml of standard zinc solution were added to separate 100 ml volumetric flasks using a calibrated burette 10 ml of the ammonium acetate solution, 4 ml of the gum Arabic solution, 45 ml of distilled water and a swirling motion are added to each flask. Add precisely 0.40 ml of the oxime solution now, then diluted it with purified water to the desired level. Shake carefully, then transfer right away to the fluorimeter's measuring cell. Set the standard using dichlorvosfluorescein solution. Measurements should be started with the most concentrated zinc solution. Compare instrument values to zinc concentrations (mg/ml).

## 11. Cadmium determination:

by using 2-(10-hydroxy phenyl benzoxazole), cadmium can be precipitated quantitatively in an alkaline solution when tartrate is present. The complex rapidly dissolves in glacial acetic acid, producing a solution with an orange hue and a brilliant blue fluorescence under ultraviolet light. The acetic acid solution serves as the starting point for the fluorometric measurement of cadmium. Use a sample solution (25-50 ml) in an aqueous solution with 0.1-2.0 mg of cadmium and around 0.1 g of ammonium tartrate. Warm to 60°C, add an equal volume of 95% ethanol, then treat with extra reagent solution the solution to 9 to 11, digest at 60°C for 15 minutes, filter, wash with 20 to 25 ml of 95% ethanol containing a small amount of ammonia; and dry the precipitate at 130°C for 30 to 35 minutes. In 50.0 ml of glacial acetic acid, dissolve the precipitate, and then check the solution's fluorescence. Check the cadmium content.<sup>[5]</sup>

## Applications in organic chemistry:

### 1. Thiamine Assay:

is measured using the blue fluorescence of trichrome, the oxidation product. An enzyme called phosphatase is used to treat the extract after the sample, such as meat or grain, has been treated with acid. In the food ingredients, phosphate esters of thiamine are present and are hydrolyzed by the latter. The first aliquot receives an oxidizing agent, such as  $K_4Fe(CN)_6$ , and the other two aliquots receive equal amounts of NaOH and isobutyl alcohol. After shaking the alcoholic layer is measured in a fluorimeter, and the aqueous layer is discarded.

### 2. Fluorometric measurement of quinine sulfate:

To create the calibration curve: Transfer a standard quinine sulfate solution (1 g/ml) in increments of 1 ml, 2 ml, 3 ml, 4 ml, and 5 ml into a series of 10 ml volumetric flasks. Dilute to the desired concentration using 0.1 N

sulfuric acid Choose the appropriate 365 nm and 459 nm emission and excitation filters and utilize a fluorometer to measure the fluorescence

### 3. For the analysis of the tablet:

Weigh 20 tablets and grind them into a fine powder for tablet analysis. In a 100 ml volumetric flask, combine 75 ml of 0.1 N sulphuric acid with a precise weight of tablet powder equal to 100 mg of quinine sulfate Filter the mixture after adding 0.1N sulfuric acid to adjust the volume to 100 ml and thoroughly mixing the ingredients to completely dissolve the medication.<sup>[5]</sup>

## Applications in Diagnosis

### 1. Human tissue laser-induced fluorescence spectroscopy for cancer detection :

Cancer has a very high occurrence and is one of the most feared diseases of our day. Early tumors frequently develop from tissue with a high rate of cell turnover and activity in healing, such as altered on the exterior of hollow organs (oral cavity digestive system, female reproductive system, etc.) Laser spectroscopic methods could be used in situ. The use of nonionizing radiation and close to real-time diagnosis guarantees that the diagnosis can be repeated lacking any negative side effects. There are two ways that Laser Induced Fluorescence (LIF) has been utilized to diagnose cancer One strategy includes injecting a medication that is specifically retained by tumors, such as hematoporphyrin derivative (HDD). Throughout the body. The medication, which is localized in the tumor, fluoresces when photoexcited with light of the proper wavelength. This tumor is detected and imaged using fluorescence. Additionally, photoexcitation causes the triplet to populate the state via a crossing of systems. The excited triplet state of the molecule might directly interact with biological components or result in the creation of harmful singlet oxygen for the host tissue. As a result, the host tissue is destroyed and used for tumor photodynamic treatment.<sup>[6]</sup>

### 2. Accurate determination of glucose:

In biological processes, glucose is regarded as a crucial part of animal and plant carbohydrates. Additionally, blood glucose levels serve as a marker for human health conditions: aberrant glucose levels reveal important details about numerous disorders such as diabetes or low blood sugar Because of its straightforward operational principles and excellent sensitivity, fluorophotometry was frequently utilized Au nanocluster stabilized by biomolecules have recently been shown to be an innovative fluorescence probe for sensitive and accurate glucose detection.<sup>[7]</sup>



## Special Application

### 1. Study of Marine Petroleum Pollutants:

One effective method for detecting oil slicks on the water's face, determining petroleum pollutants in saltwater, identifying specific petroleum deactivate compounds, and locating pollution sources is fluorescence spectroscopy main element of any hydrocarbon oil. The remaining components are mostly single-atom hydrocarbon derivatives nitrogen, oxygen, or Sulphur. Few hydrocarbons glow and the vast majority have no ability to fluoresce luminescence. Rarely does the amount of fluorescence-capable chemicals in oil bulk exceed 10%.

Additionally, petroleum strongly absorbs sunlight, notably blue and ultraviolet rays. Despite this, oil can be tested because petroleum is a luminous material and fluorescence is a phenomenon. Oils' fluorescence has a wavelength of over 260 nm and includes visible and ultraviolet spectrums. The occurrence is more common and substantial between 270 and 400 nm.<sup>[5]</sup>

#### Applications in agriculture:

Spectroscopic techniques are frequently used in agriculture, such as for identifying various crop kinds. Citrus seedling variety identification is made easy with the laser-induced fluorescence emission technique (LI FS). To distinguish between identical varieties of tea, total luminescence spectroscopy can be utilized by tea manufacturers as a rapid, inexpensive, and objective substitute for hiring trained tea tasters. Over the course of its nearly 70-year history, fluorescence spectrophotometry has developed into a method with a wide range of advanced applications. Therefore, it is important to carefully consider the best application strategy for every possible application scenario in order to get the desired results. Fortunately, there is a variety of literature and commercial solutions available for those who want to learn more about fluorescence spectrophotometry.<sup>[8]</sup>

#### Applications in Bioscience:

The high-precision measurement of DNA and RNA is one of fluorescence spectroscopy's most often-used applications in biosciences. A DNA sample is mixed with an extrinsic fluorophore, frequently ethidium bromide, and then put into a fluorescence spectrometer to measure the concentration of the material. SMRT (single molecule real-time) DNA sequencing is another contemporary use. It is anticipated that it will play a key role in the next genetic diagnostic revolution because of its capacity to manufacture long-read single molecules with great precision.<sup>[9]</sup>

#### Applications in industrial:

As a quick, noninvasive method for determining contamination, fluorescence spectroscopy is applied in a variety of industrial situations. After hydraulic fracturing for gas exploration, it has been used, for instance, to find harmful organic chemicals in groundwater.<sup>[10]</sup>

## Applications in chemical:

Fluorescence spectroscopy is used extensively in the creation of nanoparticles for possible medical applications, such as medication administration. The protein corona forms when nanoparticles are exposed to biological fluids, coating them with proteins and other biomolecules. Its safe use in vivo depends on the interactions between the nanoparticle and the protein corona. To better understand these interactions and nanostructures, time-resolved fluorescence quenching and fluorescence correlation spectroscopy are utilized.<sup>[11]</sup>

## Applications in pharmaceutical:

The pharmaceutical industry also uses spectrofluorometric methods for medication analysis. The study of jointly produced tablets that were recommended as cholesterol treatment is one example. An easy, quick, and precise method for examining the tablet area, which includes both Ezetimibe and Atorvastatin calcium, is synchronous fluorescence spectroscopy. The technique is perfect for this medication's routine quality control.<sup>[12]</sup>

## Environmental Applications:

### 1. Quantifying analytes:

- a) Insecticides<sup>[13]</sup>, antibiotics<sup>[14]</sup>, triphenyl-tin<sup>[15]</sup>, PAHs<sup>[16]</sup>, phenol and its metabolites in waters<sup>[17]</sup>
- b) PAHs in soils.
- c) Pesticides in vegetables<sup>[18]</sup>

### 2. Fingerprinting:

- (a) Seawater-dispersed fingerprinting oil.<sup>[19]</sup>
- (b) describe the phytoplankton communities in rivers and water.<sup>[20]</sup>
- (c) Using the DOM signature.<sup>[21]</sup>

I. To keep an eye on groundwater, wastewater river water, and fog water

II. To classify coastal waters and comprehend the dynamics of the mixing process between fresh- and saltwater as well as to identify sources of DOM including those that are algal derived, formed from larvaceans and copepods leached from soil and landfill leachates.

III. Researching DOM/NOM degradation in biological filtration, composting processes, effluent irrigated soils, and structural changes in DOM caused by catalytic ozonation.

IV. To investigate the biochemical oxygen demand (BOD), a physicochemical property of water. The association between microbial fluorescence and BOD is used to make this finding.<sup>[22]</sup>

V. In extracellular polymeric compounds produced by microorganisms in activated sludge and to track the dewaterability of the sludge.

### 3. To predict the chemical and microbiological composition of the soil.<sup>[23]</sup>

#### Conclusion:

A quick and sensitive technique for describing molecular environments and events is fluorescence spectroscopy. Over a fairly wide range, the fluorescence output is linearly related to sample concentration. A comparatively straightforward analytical method is fluorometry. Fluorometry is preferred over other analytical methods because of its exceptional sensitivity, high specificity, ease of use, and low cost. Usually, it has a higher sensitivity than absorbance measurements. A wide range of environmental, industrial, medical diagnostics, DNA sequencing, forensics, genetic analysis, and biotechnology applications use this highly acknowledged and effective technique. Both quantitative and qualitative analyses can benefit from using this useful analytical tool.

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#### References:

- 1) Bose, Aswathy, I. Thomas, and E. Abraham. "Fluorescence spectroscopy and its applications: A Review." *International journal of advances in pharmaceutical analysis* 8.1 (2018): 1-8.
- 2) Murthy, K. V. R., and Hardev Singh Virk. "Luminescence phenomena: an introduction." *Defect and diffusion forum*. Vol. 347. Trans Tech Publications Ltd, 2014.
- 3) Lakowicz, Joseph R., and Joseph R. Lakowicz. "Fluorescence sensing." *Principles of fluorescence spectroscopy* (1999): 531-572.
- 4) Nahata, A. "Spectrofluorimetry as an analytical tool." *Pharm Anal Acta* 2.8 (2011): 1-2.
- 5) Naresh, Kommu. "Applications of fluorescence spectroscopy." *J. Chem. Pharm. Sci* 974 (2014): 2115.
- 6) Dramićanin, Tatjana, Miroslav Dramićanin, and M. T. Stauffer. "Using fluorescence spectroscopy to diagnose breast cancer." *Appl Mol Spectrosc to Curr Res Chem Biol Sci* (2016).
- 7) Buzanovskii, V. A. "Methods for the determination of glucose in blood. Part 1." *Review Journal of Chemistry* 5.1 (2015): 30-81.
- 8) Muhammad, Mian, et al. "Spectrofluorimetric method for quantification of triazine herbicides in agricultural matrices." *Analytical Sciences* 32.3 (2016): 313-316.
- 9) Versalovic, James, Thearith Koeuth, and R. Lupski. "Distribution of repetitive DNA sequences in eubacteria and application to fingerprinting of bacterial genomes." *Nucleic acids research* 19.24 (1991): 6823-6831.

- 10) Dahm, Katharine G., et al. "Identifying well contamination through the use of 3-D fluorescence spectroscopy to classify coalbed methane produced water." *Environmental science & technology* 47.1 (2013): 649-656.
- 11) Röcker, Carlheinz, et al. "A quantitative fluorescence study of protein monolayer formation on colloidal nanoparticles." *Nature nanotechnology* 4.9 (2009): 577-580.
- 12) Miranda, Joana LA, et al. "Chip-based spectrofluorimetric determination of iodine in a multi-syringe flow platform with and without in-line digestion—application to salt, pharmaceuticals, and algae samples." *Molecules* 27.4 (2022): 1325.
- 13) Zhu, Shao-Hua, et al. "Quantitative analysis of hydrolysis of carbaryl in tap water and river by excitation–emission matrix fluorescence coupled with second-order calibration." *Talanta* 74.5 (2008): 1579-1585.
- 14) García, Inmaculada, Luis A. Sarabia, and M. Cruz Ortiz. "Detection capability of tetracyclines analysed by a fluorescence technique: comparison between bilinear and trilinear partial least squares models." *Analytica Chimica Acta* 501.2 (2004): 193-203.
- 15) Saurina, J., et al. "Determination of triphenyltin in sea-water by excitation–emission matrix fluorescence and multivariate curve resolution." *Analytica Chimica Acta* 409.1-2 (2000): 237-245.
- 16) Nahorniak, Michelle L., and Karl S. Booksh. "Excitation-emission matrix fluorescence spectroscopy in conjunction with multiway analysis for PAH detection in complex matrices." *Analyst* 131.12 (2006): 1308-1315.
- 17) Bosco, M. V., M. Garrido, and M. S. Larrechi. "Determination of phenol in the presence of its principal degradation products in water during a TiO<sub>2</sub>-photocatalytic degradation process by three-dimensional excitation–emission matrix fluorescence and parallel factor analysis." *Analytica Chimica Acta* 559.2 (2006): 240-247.
- 18) Suvadhan, K., K. Suresh Kumar, and P. Chiranjeevi. "Extractive spectrofluorometric determination of quinalphos using fluorescein in environmental samples." *Environmental monitoring and assessment* 108 (2005): 217-227.
- 19) Bugden, J. B. C., et al. "Application of ultraviolet fluorometry and excitation–emission matrix spectroscopy (EEMS) to fingerprint oil and chemically dispersed oil in seawater." *Marine pollution bulletin* 56.4 (2008): 677-685.
- 20) Berman, T., et al. "Primary production and phytoplankton in Lake Kinneret: A long-term record (1972-1993)." *Limnology and Oceanography* 40.6 (1995): 1064-1076.
- 21) Stedmon, Colin A., Stiig Markager, and Rasmus Bro. "Tracing dissolved organic matter in aquatic environments using a new approach to fluorescence spectroscopy." *Marine chemistry* 82.3-4 (2003): 239-254.
- 22) Hudson, Naomi, et al. "Can fluorescence spectrometry be used as a surrogate for the Biochemical Oxygen Demand (BOD) test in water quality assessment? An example from South West England." *Science of the total environment* 391.1 (2008): 149-158.

- 23) Rinnan, Riikka, and ÅsmundRinnan. "Application of near infrared reflectance (NIR) and fluorescence spectroscopy to analysis of microbiological and chemical properties of arctic soil." *Soil Biology and Biochemistry* 39.7 (2007): 1664-1673.

