

# INORGANIC PHOSPHATE AND PRINCIPLES OF FLUORESCENCE

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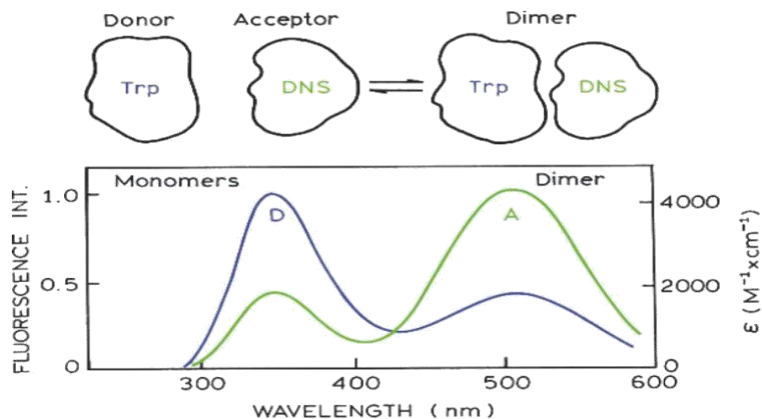
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**Abstract:** Fluorescence spectroscopy has become one of the most prevailing high-sensitivity applications for the detection and quantification of molecules. The fluorescence phenomenon studies the emission of light from excited singlet states, or the luminescence, of any molecule. These molecules absorb radiation at specific wavelengths. As this occurs, molecules become electronically excited and electrons are promoted from the ground state ( $\mu G$ ) to the excited state ( $\mu E$ ). As electrons transition back down to the ground state, energy in the form of photons is released to form immediate emissions.

**Keywords:** Förster resonance energy transfer (FRET), dihydrogen phosphate, monohydrogen phosphate, sodium 4-styrenesulfonate, Fourier-transform infrared spectroscopy (FTIR), carboxymethyl cellulose.

*Förster resonance energy transfer - FRET* Förster resonance energy transfer (FRET) is a technique that relies on the transfer of energy from a donor fluorophore to an acceptor fluorophore. The non-radiative transfer between these fluorophores is called, FRET pairs. FRET is an effective tool used to measure the distance between the donor fluorophore to that of the acceptor fluorophore. FRET occurs when the emission spectra of the donor fluorophore and the absorption spectra of the acceptor fluorophore are apart by a distance less than 10 nm and are overlapped by 30%. When a ground state acceptor fluorophore is near an excited donor fluorophore, the excited state energy is transferred. This transfer of energy leads to increased emission fluorescence intensities of the acceptor while the donor excited state lifetime and fluorescence intensity decrease. This energy transfer is a

product of dipole-dipole interactions. Alongside dipole interactions, resonance energy transfer relies on donor quantum yields, donor and acceptor fluorophore distances and spectral overlap.



**Figure. Energy transfer between tryptophan donor emission with dansyl acceptor absorption.**

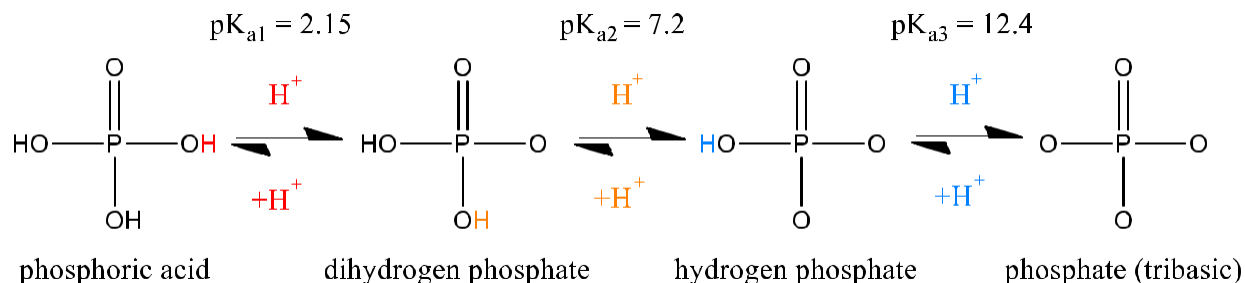
*Tryptophan (Trp) donor and dansyl group (DNS) acceptor monomers form dimers used to calculate donor-to-acceptor distances. (Copyright © Springer Nature) [1]*

### ***Fluorescence dyes for inorganic phosphate detection***

Selecting fluorophores for specific sensors requires a variety of considerations. An example would be identifying the overall net charges of prospective probes. When targeting specific molecules with anionic or cationic properties, receptors or binding sites on the fluorophore should possess high affinity for the target species. Although there are challenges to designing probes for molecular specificity, nonetheless, there have been successful methods to achieve optimized conditions for electrostatic and hydrogen-bond interactions.

In the preparation of developing an inorganic phosphate sensor, the molecular species must be understood. Inorganic phosphate has a molar mass of 94.97 g/mol. The ion forms a tetrahedral arrangement, comprising of a phosphorus central atom and four surrounding oxygen atoms. Phosphate possesses three pKa

values,  $pK_{a1} = 2.148$ ,  $pK_{a2} = 7.198$  and  $pK_{a3} = 12.35$ . Under specific pH conditions, inorganic phosphate exists as phosphoric acid, dihydrogen phosphate, monohydrogen phosphate and phosphate (tribasic).



**Figure . Molecular structures of phosphate species with  $pK_a$  values.**

**Methods: Survey for potential inorganic phosphate sensors**

*Propidium iodide and quinacrine mustard dihydrochloride hydrate spectral properties:* 15  $\mu M$  propidium iodide in tris buffer (0.1 M, pH 7) and varying concentrations of inorganic phosphate (Pi) (150  $\mu M$  – 266  $\mu M$ ) using  $K_2HPO_4$  were titrated and fluorescence emission was monitored at 632 nm upon excitation at 493 nm. The same procedure was repeated in propidium iodide 0.1% carboxymethyl cellulose (CMC) or 0.1% poly(sodium 4-styrenesulfonate (PSS)).

An identical protocol was followed for 18  $\mu M$  quinacrine mustard dihydrochloride hydrate (QM), in tris buffer (0.1 M, pH 7), 0.1% CMC or 0.1% PSS. The fluorescence emission at 500 nm was monitored upon excitation at 436 nm.

*Sephadex LH-20 for QM binding complex and spectral properties:*

Sephadex LH-20 was hydrated in MilliQ and transferred to Econo-Column® (1.5 x 10 cm, Bio-Rad, Mississauga, ON, Canada) and washed thoroughly with MilliQ. 20 mM QM was applied to the packed column. The column was washed thoroughly with 400 mL of MilliQ and was resuspended five times. 250 µL of Sephadex LH-20-QM, 500 µL of tris buffer (0.1 M, pH 7) and 100 µL of varying Pi concentrations (0 mM – 1.25 mM) were added to five separate microcentrifuge tubes (Axygen®). All tubes were vortexed for 1 minute and centrifuged at 2000 rpm. 500 µL of supernatants was monitored for QM fluorescence ( $\lambda_{ex}$ : 436 nm,  $\lambda_{em}$ : 500 nm). An identical protocol was conducted using QM (20 µM, 700 µL) and calcium phosphate ( $Ca_3(PO_4)_2$ , 0.5 g and 1.0 g) in the place of Sephadex LH-20-QM.

*Synthesis, laser ablation and FTIR of cellulose-phosphate using filter paper for binding complex:* Following pre-existing synthesis methods, 1 cm Whatman® filter paper disks were washed generously and subjected to the basic synthesis conditions for cellulose-phosphate. Controlled samples were made without the addition of sodium trimetaphosphate (STMP). Cellulose and cellulose-phosphate disks were mounted onto microscope slides and subjected to laser ablation (70 secs, 50%, 20 Hz) using PhotonMachines 193 nm short pulse width Analyte Excite excimer laser ablation system (Isomass Scientific Inc., Calgary, AB, Canada). Samples were also characterized with Fourier-transform infrared spectroscopy

(FTIR) using the Bruker Alpha FTIR Spectrometer (Plantinum-ATR attachment) (Bruker Ltd, Milton, ON, Canada).

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