Review of the relationship between the number of benzene rings within fluorescent dyes and its excitation wavelength

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Abstract. Fluorescence is part of the new biochemical technologies invented in the century. Its practical applications including fluorescence microscopy and third generation DNA sequencing technology cannot be over- looked. This review will be looking at the effects of a molecules structure on its fluorescence capabilities. More specifically, the relationship between a fluorophore's absorbance wavelength and the number of benzene rings it contains. This review has successfully used both experimental results and theoretical calculations and found a positive linear relationship between the two. This could impact chemical engineering and specifically engineer fluorophores for different situations.

Keywords: benzene rings; fluorescent dyes; fluorescent properties.

1. Introduction

Fluorescence spectroscopy is measuring light emitted from an excited molecule, which has become a better method of biological spectroscopy than its predecessors. A major advantage of fluorescence spectroscopy is its selectiveness as variables including fluorescence emission wavelength and excitation wavelength can be artificially manipulated to help distinguish analytes. The chemistry of fluorescence is used to create technology such as fluorescence microscopies, which are used to create images living cells' structures and biochemical, allowing for the visualisation of individual organelles, cells, macromolecular assemblies inside the cell and the dynamics of tissue. Furthermore, fluorescence is a part of the third generation DNA sequencing technology. Further research and development could have huge positive impact on medical and disease research[1].

The paper will be focussing on the following six linear acene molecules with increasing number of fused benzene rings from 1 to 6.

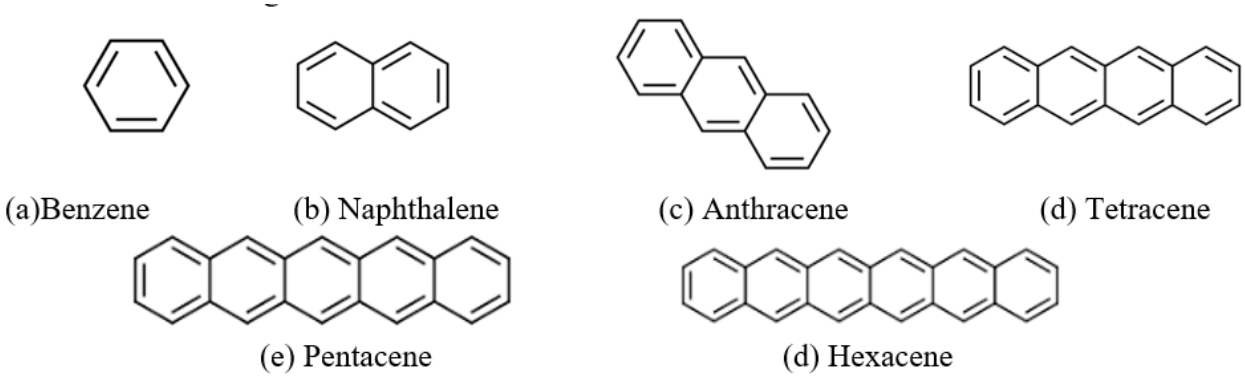


Figure 1. Molecular structures of Benzene, Naphthalene, Anthracene, Tetracene, Pentacene & Hexacene

2. Basic Principle of fluorescence mechanism

Fluorescence is a type of luminescence, the relaxation of an excited electron after energy is absorbed resulting in the emission of electromagnetic radiation. Fluorophores are molecules which get excited when energy from light or other types of electromagnetic radiation is absorbed. These molecules then emit a photon when their electrons relax to the ground state[2].

The chemical reaction behind fluorescence can be shown in the following Jablonski Diagram.

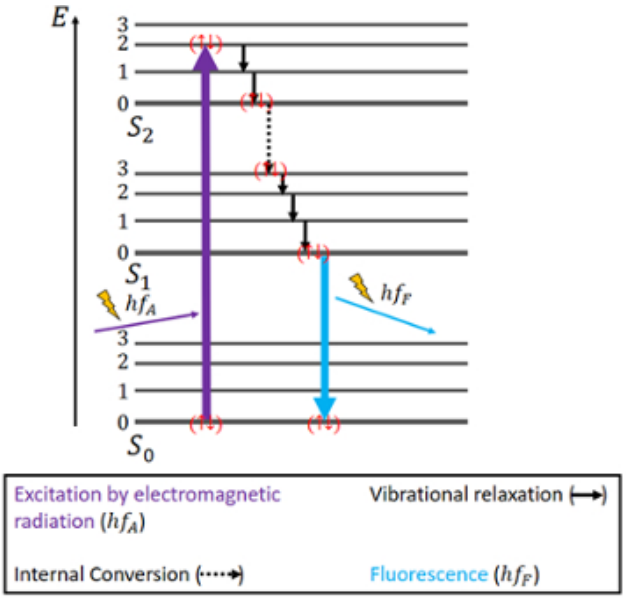


Figure 2. Jablonski Diagram of Fluorescence[2]

Figure 2 shows the electron state changes of electrons within a fluorophore when exposed to a molecular structure specific electromagnetic radiation. Using tetracene (Figure 1d) as an example, when exposed to visible light at around 450nm, which is specific to the structure of tetracene, its electrons become excited (purple arrow) and jump to the second vibrational level (V2) in the second singlet state (S2) depending on how much energy is absorbed. However, an excited electron has more energy which is unstable, therefore, it releases energy to return to its ground state. Electrons mainly loses their energy through vibrational relaxation, which refers to releasing energy into its surrounding molecules in the form of heat, represented in Figure 2 by the black arrows (V2 to V1). After reaching the lowest vibrational state (V0) within a singlet state, the electron undergoes a process called internal conversion (dotted black arrow)[3]. Internal conversion is when the excited nucleus of an atom loses energy by non-radioactively emit or eject a highly energised electron to fall to the lower singlet state. A hole in its electron shell is created due to this process, which is then filled by other electrons[4]. Internal conversion repeats until the electron loses enough energy to reach the lowest vibrational level (V0) of the first singlet state (S1*). Normally, electrons will just undergo internal conversion again to fall from first singlet state to its ground state. However, the molecule cannot complete this process by emitting another electron as it contains too much energy to be absorb by the surrounding molecules. Consequently, tetracene emits a photon (blue arrow) that is around 590nm to return to its ground state, making it a fluorophore [5].

3. Comparison between the fluorescence properties between chemicals with different number of Benzene Rings

An aromatic hydrocarbon i.e., benzene is six carbons forming a hexagonal ring with a hydrogen attached to each carbon. An aromatic hydrocarbon can either be mono, with only one benzene ring or polycyclic, multiple benzene rings fused together. The sp2 hybridised bond orbitals within a benzene allows for the existence of delocalised electrons, which requires less energy to become excited which fulfils the basic requirement for fluorescence[6]. A molecule fluoresces when the energy required to be lost for its electron to return from its first singlet state to its ground state is higher than the maximum limit for the occurrence of internal conversion. These aromatic hydrocarbons have rigid structures resulting in a large maximum permittivity value, which relates to the amount of energy the molecule can store. The rigid structure also prevents electrons from efficiently losing energy through vibrational relaxation which results in the molecule having more

energy than the limit of internal conversion, which forces it to lose energy by emitting a photon i.e., fluorescence [7].

As fluorescence depend on molecular structure, the gap between highest occupied molecular orbital (HOMO) and lowest unoccupied molecular orbital (LUMO) can be used to calculate the excitation wavelength, as it only allows a specific wavelength of light to pass through and absorbed[8]. The HOMO and LUMO refers to bonding and anti-bonding orbitals of pi bonds. Anti-bonding molecular orbitals have out-of-phase pi bond and bonding orbitals have in-phase pi bonds. The direction of the two overlapping P orbitals determines the "phase" of a pi bond. The two lobes of a P orbital can be differentiated through the positive or negative wave displacement of electrons as they are treated as mathematical waves. Parallel wave displacement will form an in-phase pi bond and non-parallel wave displacement will form an out-of- phase pi bond[9].

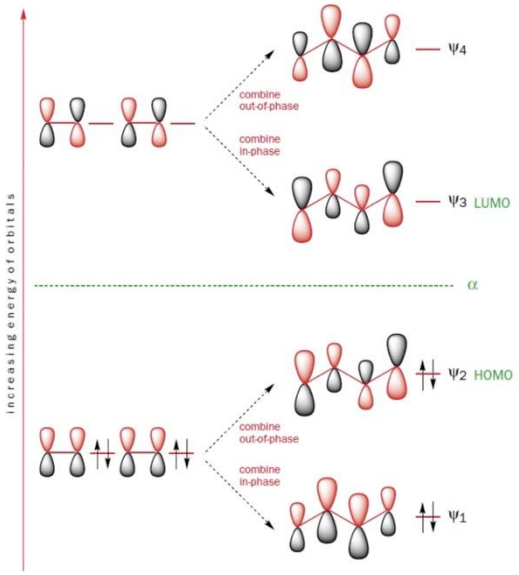


Figure 3. Butadiene (Particle in a Box Model)

Figure 3 shows butadiene with the node 2 as the HOMO and the node 3 as the LUMO. HOMO becomes the ground state as it is the bonding orbital with highest energized electrons and LUMO, the non-bonding orbital with lowest energized electrons, becomes the first singlet state (S1 from Figure 2) [10]. As the electron states are energy levels, the HOMO-LUMO gap is the energy difference between the ground and the excited electron state. Using the energy difference, the wavelength of excitation can be calculated as they should be equal after [11].

Since the excitation wavelength depends on the different energy levels in the HOMO-LUMO gap, the excitation wavelength can be manipulated by changing the molecular structure of fluorophores to increase or decrease its HOMO-LUMO gap[12]. In the case of aromatic hydrocarbons, as the number of benzene rings increase, the number of pi bonds will also increase, which causes the HOMO LUMO gap to decrease. This is because as the number of pi bond orbitals increase, the number of nodes(s) also increase, resulting in the creation of an out-of-phase node with less energy and more stability than the original LUMO nod and an in-phase node, but less stable and with more energy compared to the original HOMO node. This will lead to a decrease in the size HOMO-LUMO gap meaning the electron will need less energy to jump over the shorter energy gap, which results in longer wavelength to be absorbed[13].

To answer the research question, the theoretical model of the chemistry behind fluorescence provided in this section states that as the number of benzene rings within a fluorophore increase, the HOMO-LUMO gap decreases leading to a higher excitation wavelength. This demonstrates a positive trend between a fluorophore's excitation wavelength and the number of benzene rings it contains.

4. Benzene Rings and Excitation Wavelength

4.1 Absorption Spectrum of Benzene, Naphthalene and Anthracene

This review will be using the fluorescence data of the linear acenes in Section 1 collected in other studies. An article presented the absorption spectrum of benzene, naphthalene, and anthracene in the table below[14].

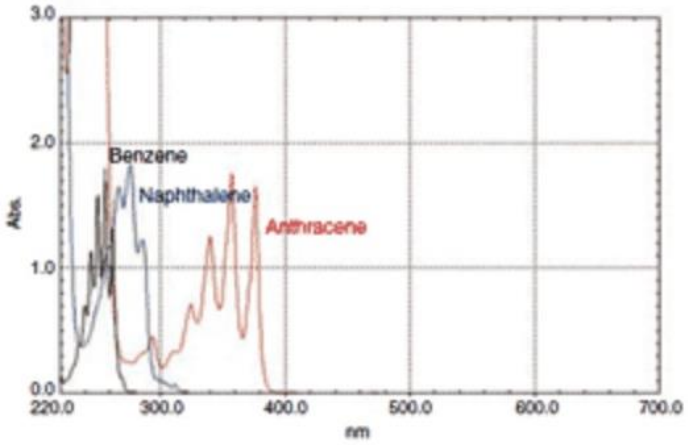


Figure 4. Absorption Spectrum of Benzene, Naphthalene and Anthracene

This article presented the h_{max} of benzene, naphthalene, and anthracene at 255nm, 286nm and 375nm respectively.

4.2 Absorption Spectrum of Tetracene, Pentacene and Hexacene

In an article published by R.Mondal included the absorption spectrum of tetracene, pentacene and Hexacene shown in Figure 5[5].

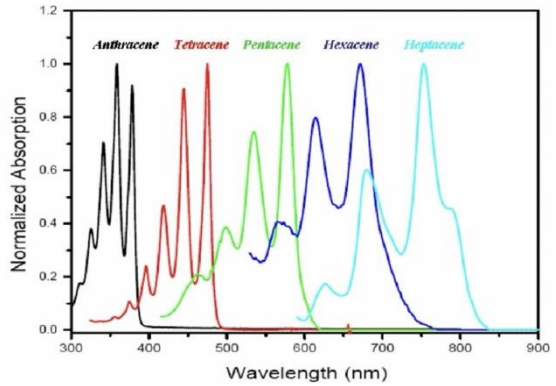


Figure 5. Normalized Absorption Spectrum of Anthracene, Tetracene, Pentacene, Hexacene and Heptacene

The article identified the h_{max} of tetracene, pentacene and hexacene to be 477nm, 582nm and 675nm respectively.

4.3 Trend Identification

A graph is then created using the data in Section D1 and D2 on Logger Pro, then applied a line of best fit to identify the relationship.

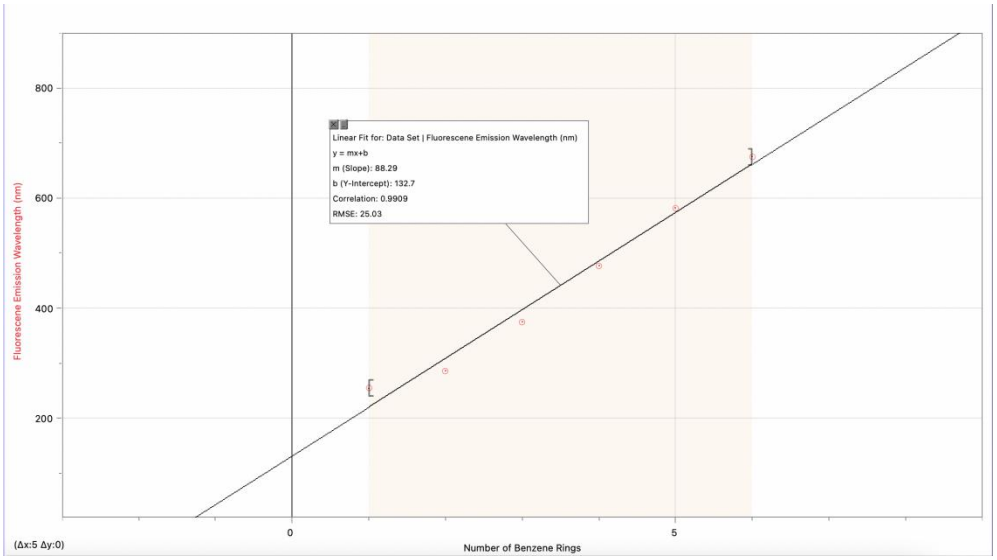


Figure 6. Experimental Fluorescence Excitation Wavelength (nm) with respect to Number of Benzene Rings

In Figure 6, a line of best fit can be seen with the equation fluorescence excitation wavelengths equals 88.29 times the number of benzene rings plus 132.7. The positive gradient describes the positive relationship between the experimental fluorescence excitation wavelength (nm) with respect to number of benzene rings and introduces the linearity between them.

4.4 Evaluation

Although, the results seem accurate and reliable, many errors could still exist within the data presented in Sections 4. First, the data for the linear acenes came from two different sources which could lead to many errors. Although differences in method have little to no effect on the excitation wavelength of these molecules, the differences in spectrophotometers could cause deviation between the results. The article presented in Section 4.1 used a Shimadzu UV-2550 UV-VIS spectrophotometer and the article presented in Section 4.2 used a UH4150AD UV-Vis-NIR Spectrophotometer. The difference in spectrophotometers could have different error margins for wavelength collection causing the wavelength data to be inaccurate. However, both articles stated that error with regards to controlled variables were kept to a minimum allowing the results to be reliable. Therefore, the trend established in Section 4.3 can be considered reliable.

4.5 Fluorescence Emission Spectrum of Benzene and Anthracene

The trend established in Section 4.3 can also be seen in the data collected by a team from Tianjin University in China. The article is written in Chinese on collected experimental emission spectrum for benzene, naphthalene, and anthracene, however, only the emission spectrum of benzene and anthracene is included in Figure 7 below[15].

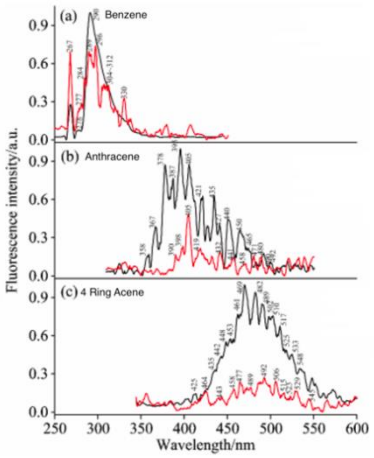


Figure 7. Fluorescence Emission Spectrum of

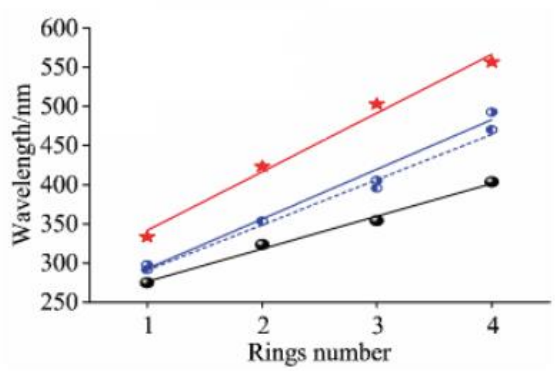


Figure 8. Trend identified between the Number of Benzene and Anthracene rings and Emission Wavelength

The red curve on graph (a) and (b) in Figure 7 is the fluorescence emission spectrum of benzene and anthracene. The article presented h_{max} of benzene and anthracene as 296nm and 405nm respectively. The article also included a graph (Figure8) showing the relationship between the fluorescence emission wavelength and the number of benzene rings.

The two blue curves in Figure8 exhibits a clear positive linear trend between the fluorescence emission wavelength and the number of benzene rings, which implies a similar relationship between the number of benzene rings and the excitation wavelength. This article presents the same experimental relationship as and Section 4.3. This allows the positive linear trend between the number of benzene rings within a fluorophore and its excitation wavelength to be consider accurate and reliable.

5. Conclusion

This review introduces the basic mechanism behind fluorescence and compared fluorophores with different number of benzene rings. By reviewing the experimental data from various sources in Section 4, it can be said that there is a positive linear trend between increasing the number of benzene rings and the excitation wavelength which is seen in Figure 6 and Figure 7B.

After accessing the applications of this technology, future research in this field should be prioritized. Its capabilities in biochemistry such as DNA sequencing and cell imaging is far beyond other current technologies. Although, current researchers already use fluorescence technologies, it is far from its full potential. For example, new technologies could be created for plant cell imaging as there are still many gaps in the field[16].

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