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Auramine-O dye abnormal Stokes shift in aqueous solution due to J-aggregate formation

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ABSTRACT

Auramine-O (Au-O) dye is an imine-type colorant used in various industries such as food, textiles, leader, printing, optics, biomedicine, lasers, fluorescent microscopy and more. In this work, we found that Au-O dye forms J-aggregate macromolecules in very acidic aqueous solutions (pH=0.5) and high concentrations (10^{-2} M), resulting in a red-colored solution with a λ_{max}=295 nm and a shoulder peak at 537 nm in the UV-visible spectrum. This J-aggregate exhibited fluorescence emission at λ_{max}= 415 nm, a previously unreported finding. The 120 nm Stokes shift for the J-aggregate suggests that this dye may be suitable for fluorescent microscopy without the issues of low signal-to-noise ratio and self-quenching. It was shown that applying a magnetic field has no effect on the emission of this dye. Finally, the Michler's ketone resulting from the Stephen's reduction reaction also emits fluorescence, but due to internal rotation, it cannot be replaced by Au-O.

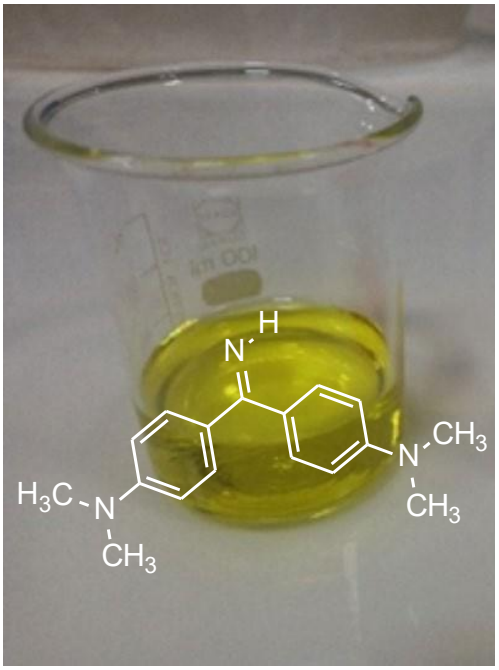
KEYWORDS: *Auramine-O; J-aggregate; Stokes shift; Red-Edge effect; fluorescent microscopy*

Introduction

Auramine-O (Au-O) is a cationic dye (shown in Scheme 1) which is used for dyeing textiles, leather, food, paints, dye components in inking ribbons, carbon paper, ballpoint pastes, paper dyeing, flexographic printing, host-guest studies, fluorescent microscopy, as a fluorescence marker for detecting of amyloid fibrils, and for adsorption on insulin fibrils[1-5]. In its pure form, Au-O is a solid that forms yellow powder or needle crystals. It is highly soluble in water and also soluble in ethanol or diethyl ether. Au-O is a toxic dye and very resistant in the environment. Therefore, many attempts have been made to degrade it [6,7] but this is not what we are looking for in this study.

On one hand, fluorescent dyes are utilized in a variety of in vitro biological applications due to their optical and structural stability, non-invasive therapeutics properties, cell compatibility, and real-time response. However, the most commonly used fluorescent dyes, including fluorescein, Rhodamine, Oxazine, and Cyanine, have small Stokes shifts of less than 30 nm. This results in poor signal-to-noise ratios and self-quenching. For next-generation applications, such as single-molecule analysis, fluorescent dyes with large and tunable Stokes shifts are necessary for precise imaging and sensing[8]. Various strategies have been developed to achieve this by making structural changes to the fluorescent dye molecule[8]. This method has the problem that structural changes in molecules are tedious and time-consuming, and an easier method must be sought. One of the easiest methods is to change the pH or concentration of the dye solution and cause the molecules to aggregate. But, the effect of dye molecule aggregation on the Stokes shift for use in fluorescent microscopy has not been reported, so far.

Au-O is another fluorescent dye that can be



Scheme 1. Chemical structure of Au-O with Canary yellow color in water.

effectively used to detect acid-fast bacteria with fluorescent microscopy[9]. However, due to molecular rotation, its fluorescence is weak. Molecular aggregation refers to the formation of large clusters of molecules due to external stimuli like changes in pH or viscosity, as well as micelle formation, among others[5]. Aggregation is a common occurrence with significant effects on the photo chemical, physical, and biomedical properties of materials [1-5]. In certain applications, such as analytical analysis, aggregation can lead to undesired effects and should therefore be avoided[10-15]. However, in cases like singlet fission in photovoltaic cells, aggregation can prove to be beneficial[1-5]. To date, no aggregation of Au-O has been reported in aqueous solutions. Nonetheless, Oster and Nishijima serendipitously observed observable fluorescence in high viscosity glycerin mediums[15].

In this work, an attempt has been made to investigate the effect of molecular aggregation on the Stokes shift for use in fluorescent microscopy. Furthermore, there are many unanswered questions about Au-O dye′s behavior in aqueous solution. To address these questions, this study re-examined the absorbance, fluorescence, and aggregation behaviors of Au-O dye in different pH levels and concentrations in aqueous solution.

Experimental and materials

4-[4-(dimethylamino) benzenecarboximidoyl]-*N*,*N*-dimethylaniline hydrochloride; (Au-O), $SnCl_2$, NaOH, HCl, H_2SO_4 and HNO_3 were purchased from Sigma-Aldrich. All materials used were of analytical grade. Solutions with varying pH levels were prepared by adjusting with NaOH and HCl solutions. Dilute solutions were also prepared by serial dilution of the stock solution. UV-visible measurements and photoluminescence (PL) spectra recorded using a Biochrom Biowave series and JASCO FP-6500 instruments, respectively. Quartz cuvettes were utilized for spectrophotometric and PL measurements. The handheld magnet used had magnetic field strength of 25 mT.

Results and discussion

The pH dependence of color of Au-O

Fig. 1 shows the pH dependence of the color of 1×10^{-3} M Au-O aqueous solution, ranging from pH=3 (left) to pH=10 (right) in one-step pH increments. The solutions were stored in a room under normal sunlight conditions. It can be observed that the color of Au-O is colorless at pH=3 and 4, canary yellow at pH=5 to 7, and colorless again at pH=8 to 10. After one week, all Au-O solutions became colorless. Similar to the aromatic amine chemical class, Auramine hydrochloride may be susceptible to photolysis or photo-oxidation through hydroxyl and peroxide radicals in water, or photo-degradation on the surface of glass containers.

Fig. 2 displays the UV-vis. spectra of an Au-O (1×10^{-3} M) aqueous solution at various pH levels, while Fig. 3 illustrates the pH dependence of the Au-O aqueous solution′s UV-vis. spectra band maximum within the 300 nm to 500 nm range with an S-shaped pattern and a inflection. It is evident that as the pH of the solution increases, the maximum absorbance within 300 to 450 nm range also increases.

Fig. 4 displays the PL spectra of an Au-O (1×10-3 M) aqueous solution at different pH levels, while the Fig. 5 illustrates the pH difference of the PL spectra within the 350 nm to 600 nm range, focusing on the band maximum of fluorescence. It is evident that an increase in the solution′s pH results in a higher maximum PL intensity. Another factor contributing to the varying intensities is the discrepancy in absorbance at the excitation wavelength of 310 nm.

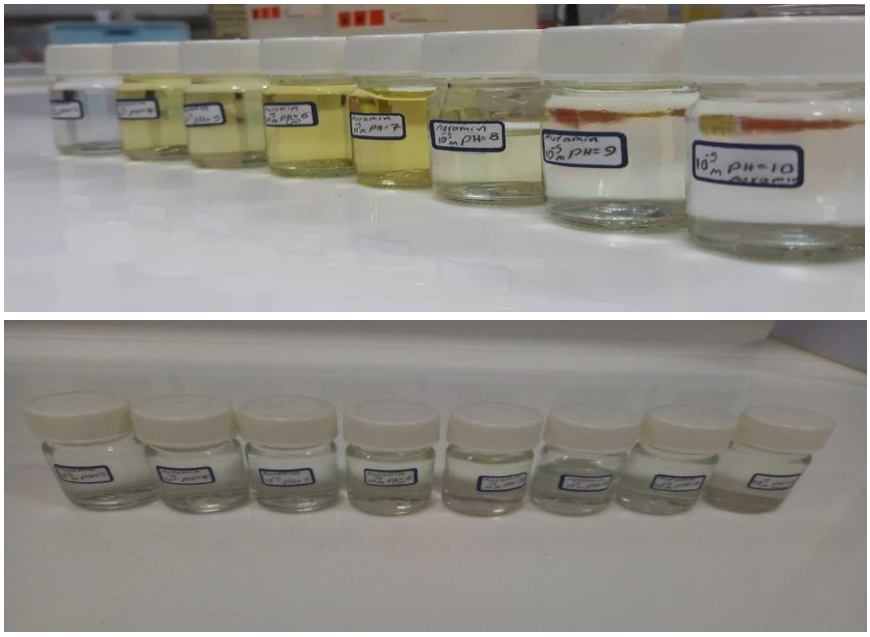


Fig. 1. The pH dependence of the color of Au-O aqueous solution (1×10-3 M) from pH=3 (left) to pH=10 (right) with a one-step pH increment (top) and their photo-degradation on the surface of glass containers (down).

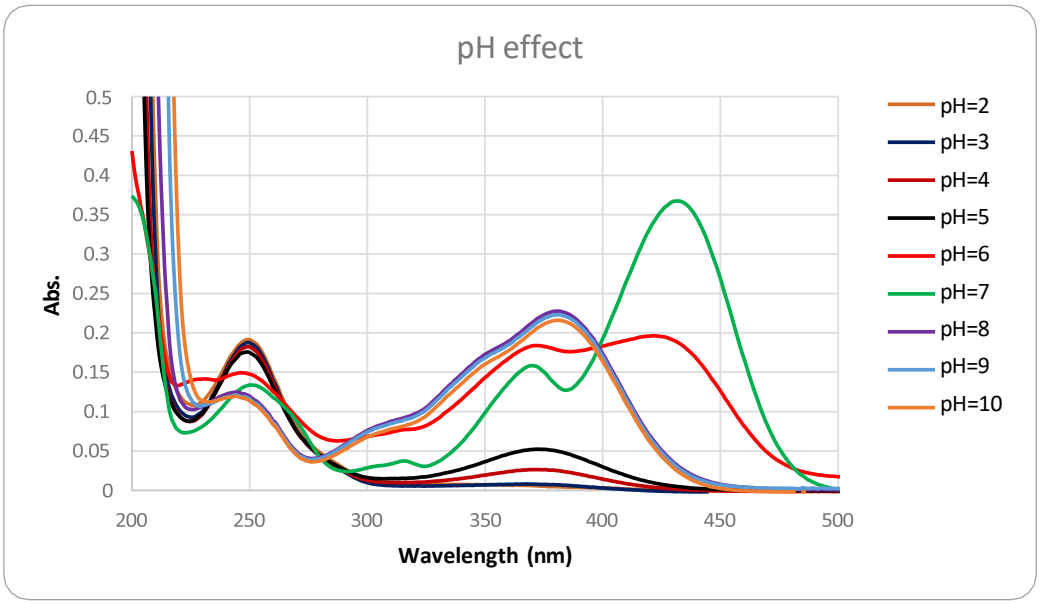


Fig. 2. The UV-vis. spectra of an Au-O (1×10-3 M) aqueous solution at various pH levels.

The results show that Au-O (I) has a pH dependent spectra. The typical spectrum changes occur above pH 6.0, decrease in absorbance at 370 nm and an increase in fluorescence emission intensity at 405 nm. These changes can be explained by assuming the formation of an Au-O amine structure (II) through the hydroxylation of Au-O in alkaline pH, as shown in Scheme 2.

As can be deduced from this mechanism, in acidic solutions, the imine double bond is broken. This results in the rotation around the carbon-nitrogen bond being added to the rotations of the molecule. Therefore, the rigidity of the molecule is reduced, decreasing its fluorescence emission. Conversely, this does not occur in alkaline environments. As a result, the fluorescence emission of the Au-O molecule increases.

In the neutral Au-O (I) molecule, rotation around the bonds at the site indicated on the NMR scale is easy and helps balance the ring protons. The rotation at the phenyl ring junction is illustrated in Scheme 2 (A). However, as the pH of the solution decreases and the imine nitrogen atom becomes protonated, the involvement of the nitrogen atoms in the resonance transfer along the phenyl ring and the formation of state Scheme 2 (B) occurs. This slow down and eventually stops the phenyl rotation, which can impact the fluorescence of the molecule at various pH levels in the solution, leading to a decrease in the fluorescence effect. These processes are depicted in Scheme 2.

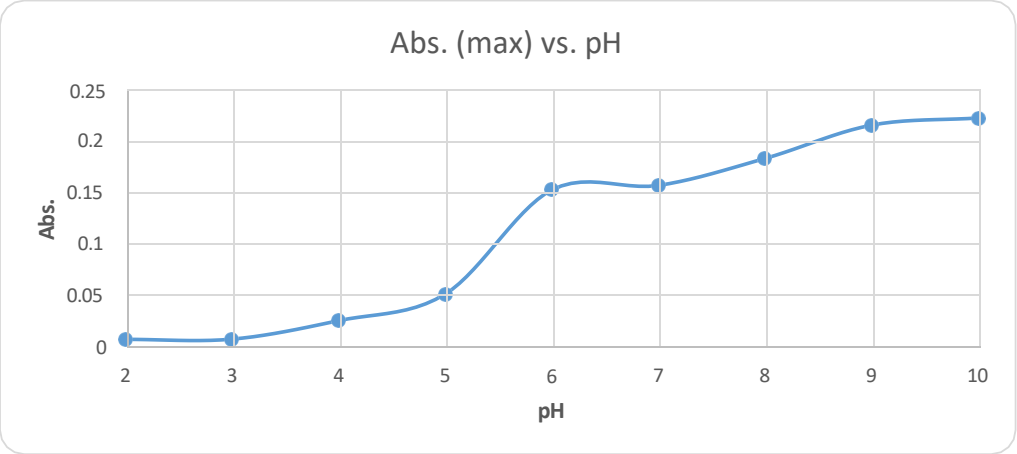


Fig. 3. The pH dependence of UV-vis. spectra absorbance maximum of Au-O (1×10-3 M) in an aqueous solution.

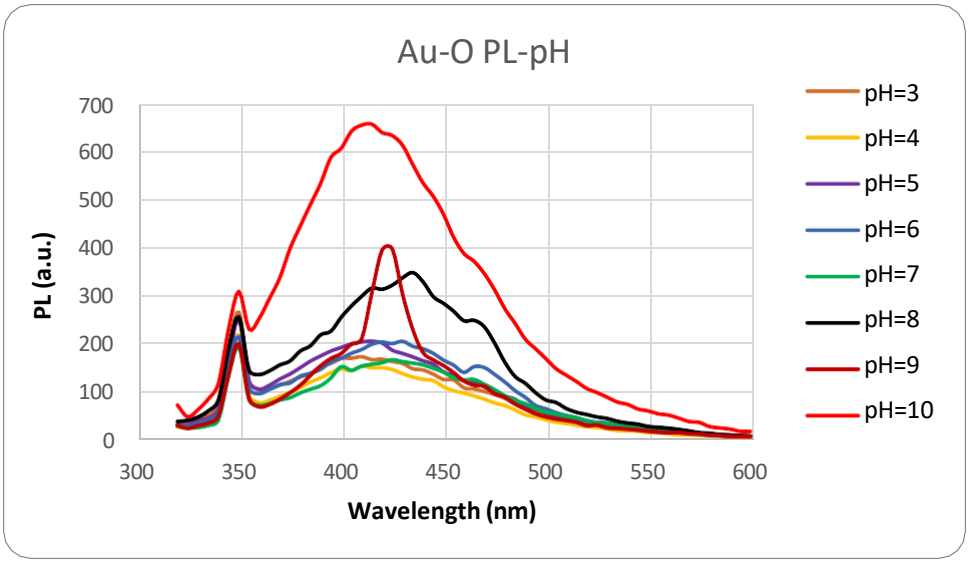


Fig. 4. The PL spectra of an Au-O (1×10-3 M) aqueous solution at different pH levels, with an excitation wavelength of 310 nm.

Isosbestic point calculation

For the reaction in Scheme 2, the absorption spectra for the pH gradient should have an isosbestic point[16], as shown in Fig. 6. Based on the second method mentioned in reference[17], the pK_a and pK_b of the Au-O dye can be roughly calculated using the absorbance data at two maxima such as 380 nm and 430 nm, in terms of pH through equations 1 to 6. This leads to pKa=5.9 and pK_b=8.1, respectively. The isosbestic point is very

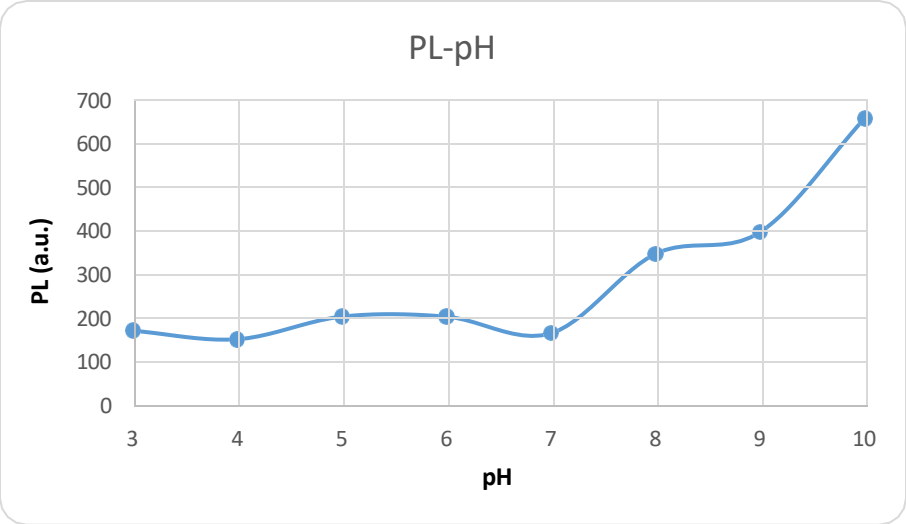
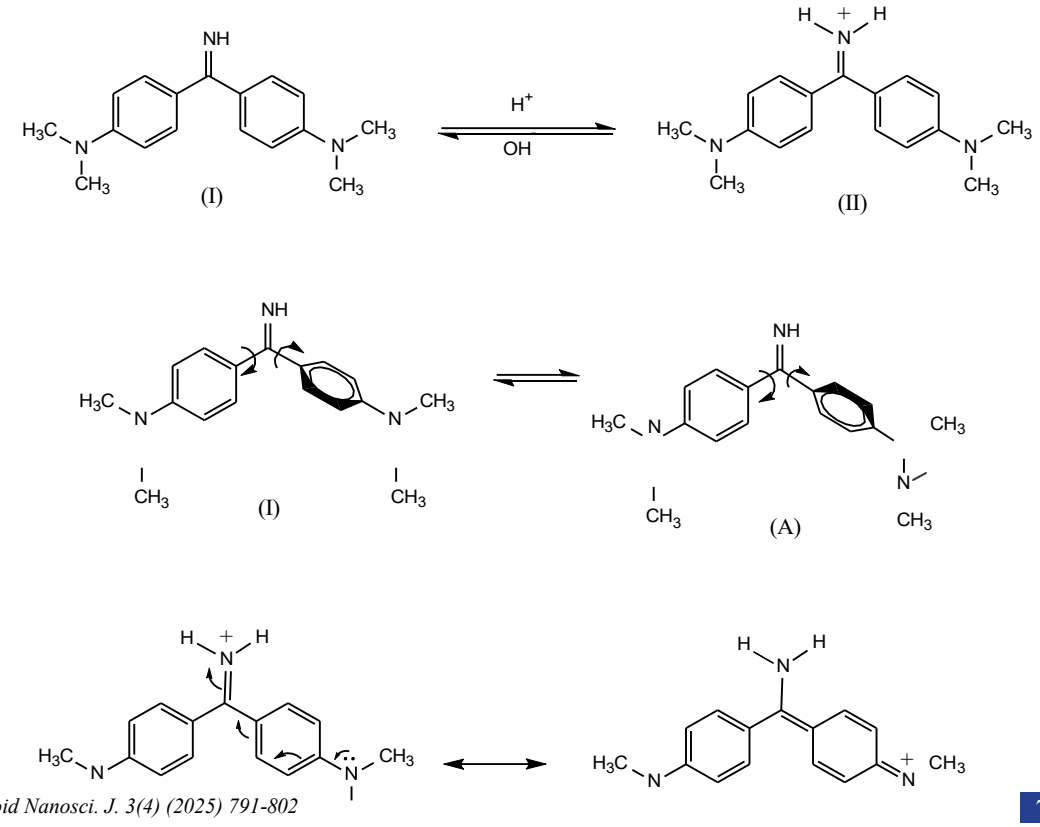


Fig. 5. The pH dependence of PL spectra maximum of an Au-O (1×10-3 M) aqueous solution with an excitation wavelength of 310 nm.



Scheme 2. Proposed mechanism for pH effect on Au-O dye solution florescence.

important, such as: Firstly, in chemical kinetics, isosbestic points are used as reference points in the study of reaction rates, as the absorbance at those wavelengths remains constant throughout the whole reaction. Secondly, in clinical chemistry, as a quality assurance method, to verify the accuracy in the wavelength of a spectrophotometer. This is done by measuring the spectra of a standard solution at two different pH conditions (above and below the pKa of the substance). This point is not seen in non-colorful solutions.

$$-0.0159x + 0.1783 = 0.0598x - 0.2686 \quad \textbf{(1)}$$

$$\rightarrow 0.1783 + 0.2686 = 0.0598x + 0.0159x \quad \textbf{(2)}$$

$$\rightarrow 0.4469 = 0.0757x \quad \textbf{(3)}$$

$$\rightarrow x = \frac{0.4469}{0.0757} = 5.9 \Rightarrow pk_a = 5.9 \quad \textbf{(4)}$$

$$\rightarrow pk_a + pk_b = 14 \quad \textbf{(5)}$$

$$\rightarrow pk_b = 14 - pk_a = 14 - 5.9 \Rightarrow pk_b = 8.1 \quad \textbf{(6)}$$

Aggregation

Fig. 7 displays UV-visible spectra of Au-O in aggregate form in a highly acidic aqueous solution (pH=0.5) with a local λ_{max}=537 nm and varying concentrations. The curve labeled C in Fig. 7 is a consequence of the high concentration and extremely high extinction coefficient of Au-O, leading to absorption that falls outside of the detection range and appears as a plateau or flat line.

In Fig. 7, the absorption spectrum at a concentration of 5×10^{-2} M shows a straight line in the 300-450 nm range. This straight line can be explained by the Franck-Condon principle, which describes the intensity of vibronic transitions. When the inter-nuclear distances in the upper and lower states are approximately equal (re" ≈ re'), the most likely transition is (0, 0). However, there is a non-zero probability of (1, 0), (2, 0), (3, 0) etc. transitions. These successive lines will have rapidly diminishing intensities, resulting in a straight line in the absorption spectrum.

Fig. S1-a, found in the supporting information section, displays the Au-O colorant in a neutral solution (yellow) and in aggregate form in an acidic solution (red). Fig. S1-b, also located in the supporting information section, demonstrates aggregation only in HCl and H_2SO_4, as it does not occur in HNO_3 due to oxidation or decomposition in this acid, as evident from its color.

Fig. S2 shows transitions based on Kasha's excitation theory[18-22] for H and J aggregate molecules. From Figs. 8 and 11 as well as this theory, it can be concluded that the Au-O dye undergoes a J-aggregate form in a very acidic (pH=0.5) and concentrated (3×10^{-2} M) aqueous solution.

It is worth noting that Kasha's excitation theory discusses the emergence of new electronic transition bands in the solution of dyes or certain chemical compounds under the influence of intermolecular forces such as hydrogen bonds. The theory also explains how these molecules can be attached together in two forms: (i) they are sandwiched on top of each other, referred to as H-aggregate

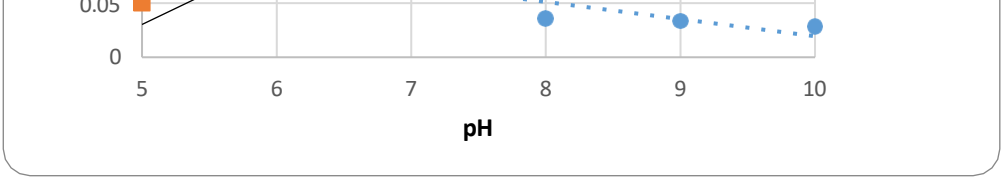


Fig. 3. The pH dependence of the absorbance spectra of an Au-O (1×10-3 M) aqueous solution at two maxima of 380 and 430 nm.

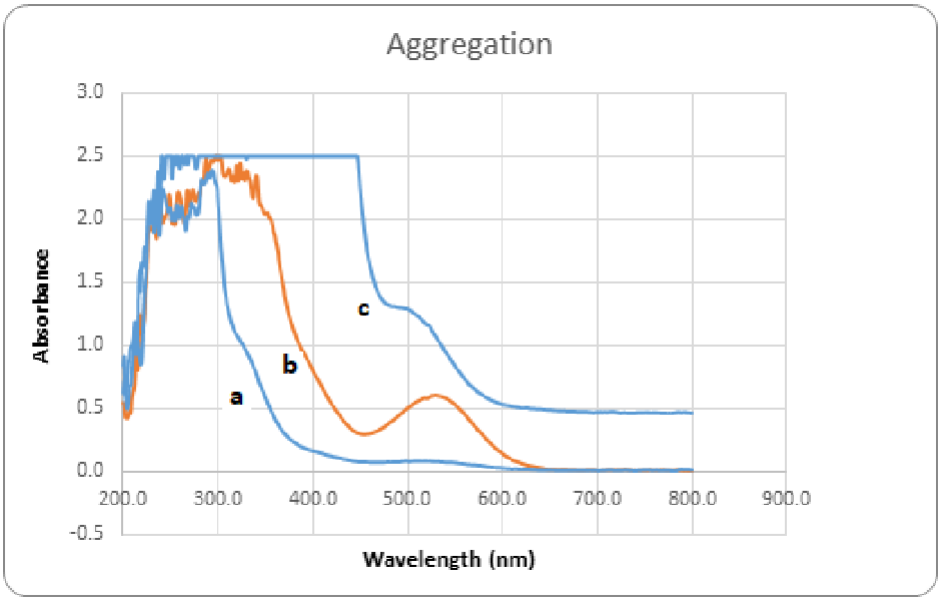


Fig. 7. The UV-vis. spectra of Au-O in aggregate form in an acidic aqueous solution;

(resulting in a blue shift) and (ii) they stick together head to tail, referred to as J-aggregate (resulting in a red shift with respect to its original band), as shown in Fig. S2 (in the supporting information section).

It should be noted that, contrary to expectations, the color change due to aggregation for this dye is very instantaneous. As shown in Fig. S3 (in the supporting information section), it is only necessary for the concentration to be very high and the pH to be very low. In such cases, the red color appears quickly; otherwise, aggregation is not possible.

Photoluminescence

Fig. 8 shows UV-vis. and PL spectra of the Au-O colorant in a neutral solution (pH=6). Au-O exhibits a weak photoluminescence. This is because Au-O is a molecular rotor, leading to efficient non-radiative intramolecular torsional relaxation of the excited state, resulting in extremely weak fluorescence emission in its monomeric form in dilute solutions. However, upon aggregation (refer to Fig. 9), these relaxation processes are significantly hindered, causing fluorescence emission in these aggregate species.

Fig. 9 shows the photoluminescence spectrum of the Au-O J-aggregate in an acidic H_2O solution (pH=0.5). It exhibits a pronounced photo emission, with fluorescence emission at a local maximum of λ_{max}=415 nm. This molecular J-aggregation demonstrates that aggregation can occur in aqueous solutions with high concentrations (1×10^{-2} M) and low pH values (0.5), or in highly viscous media as reported by Oster and Nishijima[15]. The Stokes shift is defined as the difference between the positions of the highest band maxima of the absorption spectrum and the highest band maxima of the emission spectrum. In the aggregated form (see Fig. 9), the positions of the highest band maxima of absorption are at 295 nm and of emission at 415 nm, resulting in a Stokes shift of 120 nm (equivalent to 83333.3 cm^{-1}), which is a significantly large and abnormal Stokes shift. Moreover, in this solution, fluorescence quenching occurs where the fluorescence of the molecule is extinguished by chloride ions with a high concentration.

Deuterium isotopic effect

Large Stokes shifts are categorized as follows:

- ✓ Greater than 70 nm usually indicate photo-induced excited state inter or intramolecular proton transfer.
- ✓ Stokes shifts in the ranges of 50 to 70 nm wavenumbers can be attributed to changes in the dipole moment in polar solvents.
- ✓ Purely vibrational changes resulting from

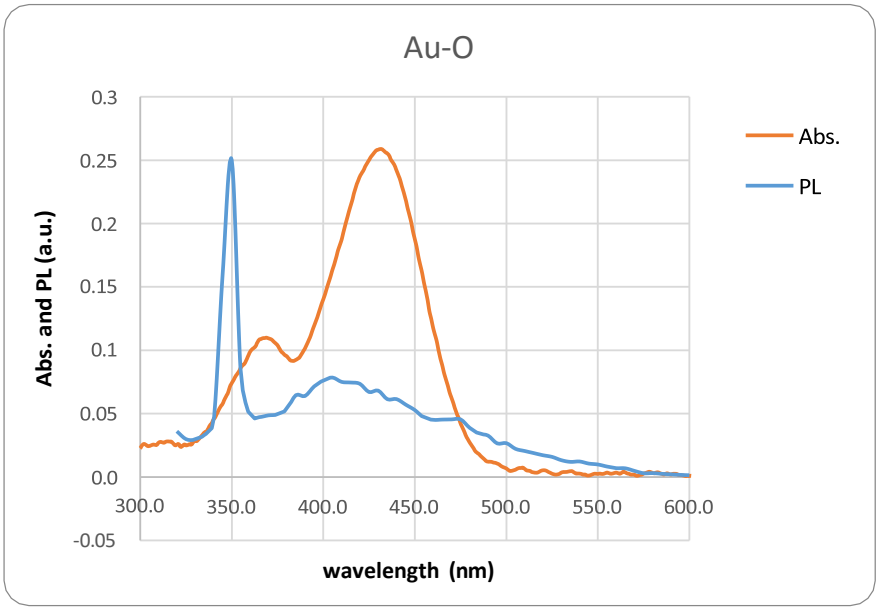


Fig. 8. The UV-vis. and PL spectra of Au-O in neutral solution (1×10-3 M, pH=6) with yellow color.

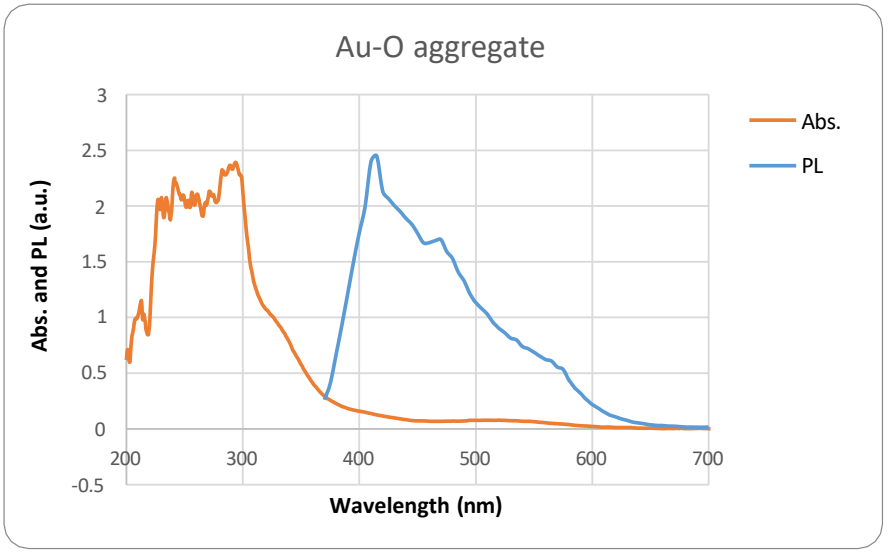


Fig. 9. The UV-vis. and PL spectrum of the Au-O aggregate in an acidic aqueous solution (1×10-2 M, pH=0.5) with red color.

the shift of the potential energy curve in the excited state compared to the ground state are typically relatively small.

When considering a Stokes shift of 120 nm, the deuterium isotope effect on fluorescence can be utilized to confirm proton transfer in the excited state.

Fig. S4 in the supporting information section displays images of Au-O aggregates in ordinary water (H_2O) and heavy water (D_2O; 3×10^{-2} M, pH=0.5). Fig. 10 presents their Absorbance and PL spectra with 350 nm excitation. The fluorescence of Au-O aggregate in heavy water is noticeably different and stronger compared to ordinary water, indicating a proton transfer in the excited state. If the Stokes shift was anticipated to be due to a change in the solution′s acidity, we would have expected to observe a shift from pH=3 (with the

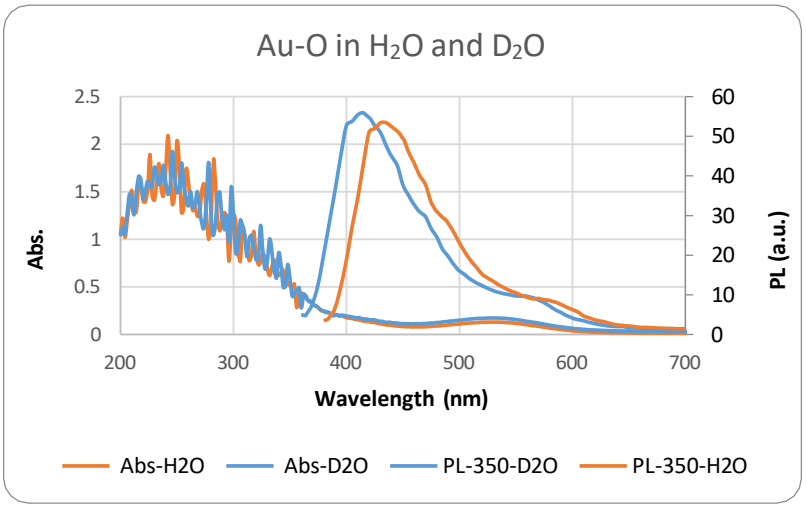


Fig. 10. The absorbance and PL spectra of Au-O aggregates in H2O and D2O (3×10-2 M, pH=0.5).

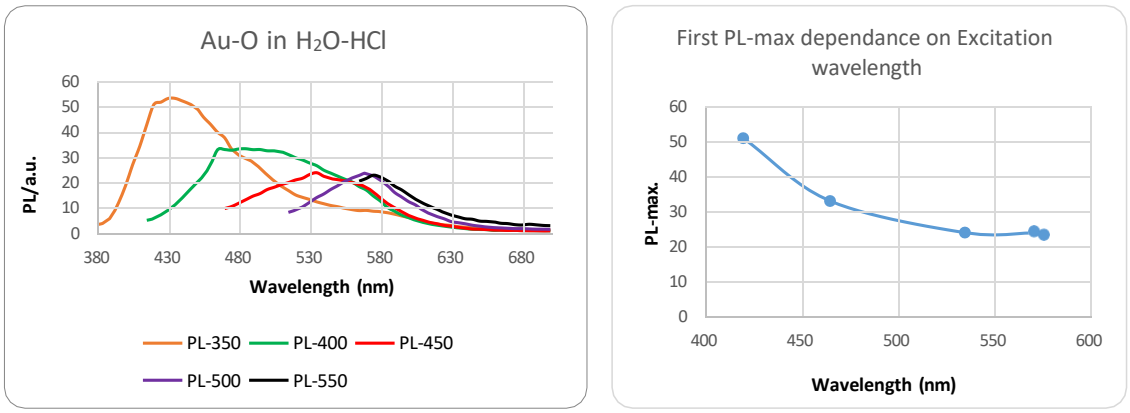


Fig. 11. -a. PL spectra of an Au-O aggregate in regular water (3×10-2 M, pH=0.5) as well as movement of the PL maximum.

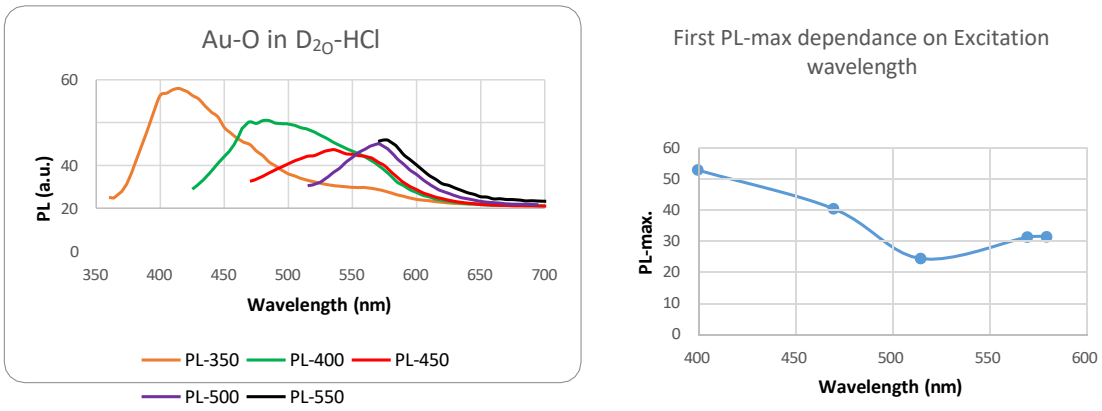


Fig. 11. -b. PL spectra of an Au-O aggregate in heavy water (3×10-2 M, pH=0.5) as well as the movement of the PL-maximum.

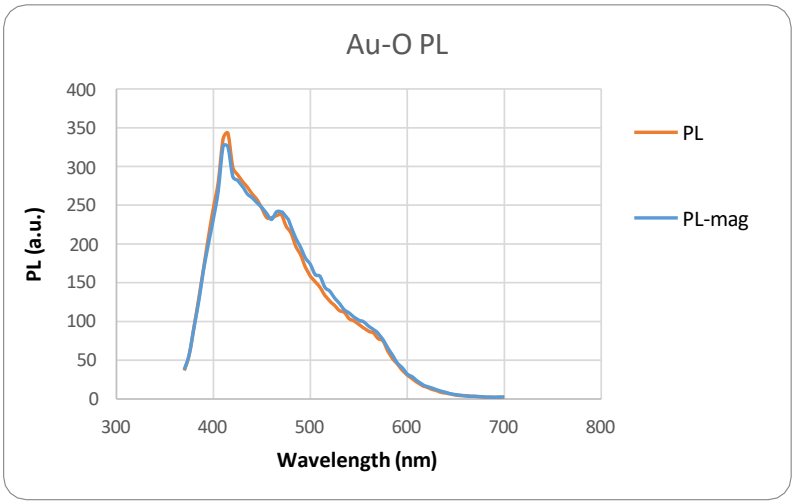


Fig. 12. The PL spectrum of Au-O in water was measured both in the presence and absence of a handheld magnet (25 mT).

PL maximum at 413.3 nm) to pH=0.5 (with the PL maximum at 410.0 nm). However, this shift was not observed, suggesting that it is likely associated with an isotopic effect.

The Red-Edge effect

Figs. 11-a and 11-b show photoluminescence (PL) spectra of Au-O J-aggregate in regular water (H_2O) and heavy water (D_2O) with varying excitation wavelengths. It is evident that the maximum PL spectrum and shape depend on the excitation wavelength. This behavior is known as the Red-Edge effect. For instance, the peak fluorescence position of graphene oxide (GO) in a polar solvent is greatly influenced by the excitation wavelength. Researchers have found that the strong excitation wavelength-dependent fluorescence in GO stems from the "giant red-edge effect"[23]. The Red-Edge effects sever as valuable tools for examining the mechanisms of different excited-state reactions, including isomerizations, electron and proton transfers, and fluorescence resonance energy transfers.

Since visible light waves in the red wavelength region (620 to 670 nm) more penetrate tissues, it is excellent to have a dye that can exhibit dependent fluorescence emission with excitation wavelength[24]. Au-O j-aggregate was capable of exhibiting excitation dependent fluorescence, which is widely known as the red edge effect, with a large Stokes shift above 120 nm, making it capable of accessing the red wavelength region of the spectrum.

Photoluminescence under magnetic field

Fig. S5 (found in the supporting information section) displays the set up for photoluminescence measurements, including the use of a handheld magnet (25 mT). Fig. 12 shows the PL spectra of Au-O J-aggregate form in an acidic aqueous solution with and without the presence of a handheld magnet. It is evident that the fluorescence of the Au-O aggregate remains unchanged in the presence of a magnetic field. This experiment was conducted because certain diimines (not discussed here) or other compounds[25] may exhibit changes in fluorescence intensity or its maximum location (finally result in Stocks' shift value change) in the presence of an external magnetic field.

Stephen's reduction reaction of Au-O

The Stephen′s reduction reaction is a chemical reaction that involves the conversion of a nitrile to its corresponding aldehyde or ketone in the presence of $SnCl_2$ and HCl. This reaction is primarily an organic redox reaction. The key steps in this reaction mechanism involve the reduction of nitriles to imines with the aid of $SnCl_2$ and HCl. After this, the resulting iminium salt must be hydrolyzed using water and heat to form the corresponding aldehydes or ketones along with ammonium chloride and $SnCl_4$[26]. Since Au-O is an imine type canary yellow dye, it can be reduced using the Stephen′s reduction reaction. Scheme 3 (in the supporting information section), shows the chemical reduction of Au-O to the corresponding

Michler′s colorless ketone via the Stephen′s method. The Michler's ketone shows an absorption maximum of 365 nm and emission at about 500 nm (not shown here). Although this ketone has a good Stokes shift, but it has weak fluorescence due to internal rotations. Furthermore, this test was performed because some diimines (not reported here) can be reduced in this test, causing the oxidation of Sn (II) to Sn (IV) and changing their emission region due to the formation of a new complex between Sn (IV) and the obtained ketone or aldehyde. As can be seen, this did not happen in this case.

Conclusion

From the results obtained in this work, it can be concluded that:

- Au-O dye forms J-aggregate macromolecules in very acidic aqueous solutions (pH=0.5) and high concentrations (10^{-2} M), resulting in a red-colored solution with a λ_{max}=295 nm and a shoulder peak at 537 nm in the UV-visible spectrum.
- This J-aggregate exhibited strongly fluorescence emission at λ_{max}= 415 nm, a previously unreported finding.
- The 120 nm Stokes shift for the J-aggregate suggests that this dye may be suitable for fluorescent microscopy without the issues of low signal-to-noise ratio and self-quenching.
- It was shown that applying a magnetic field has no effect on the emission of this dye.
- The Michler′s ketone resulting from the Stephen′s reduction reaction also emits fluorescence, but due to internal rotation, it cannot be replaced by Au-O.
- The final conclusion is that molecular rotors, such as Au-O, exhibit extremely weak fluorescence emission in their monomeric form in dilute solutions due to an efficient non-radiative intramolecular torsional relaxation of the excited state.

To overcome this problem four strategies can be used:

- Aggregation in viscose solutions
- Aggregation through hydrogen bonding in highly acidic and concentrated solutions
- Trapping guest molecular rotors in caged host supra-molecules via host-guest solutions
- Immobilizing of molecular rotors on fibers

Additionally, due to its acidic and basic properties, this molecule is capable of undergoing acid and base reactions in the presence of light and facilitating proton transfer in the excited state. This is associated with an exceptionally high Stokes shift that has not been reported previously.

Acknowledgements

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Author contributions

All chemical reactions were conducted by G. Darvishzadeh while pursuing their Ph.D. degree. N. Samadi and A. Hassanzadeh supervised the study, designed the report, and interpreted the results. Additionally, G. Marandi as an advisor of this research work contributed to interpreting the results and to the writing and editing of this paper.

Data availability

Data is provided within the manuscript or supplementary information files.

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