ADDIS ABABA UNIVERSITY
SCHOOL OF GRADUATE STUDIES
DEPARTMENT OF CHEMISTRY

Tautomersim and Spectral Properties of Alizarin (1,2-Dihydroxyanthracene-9, 10-Dione)

Advisor: Dr. Mesfin Redi

By: Tesfaye Kebede Dabsu

A Project Submitted to the School of Graduate Studies in Partial Fulfillment of the Requirements for the Degree of Master of Science in Chemistry

July, 2007

I. Acknowledgements

I would like to express my sincere feelings of gratitude and appreciation to my advisor, Dr. Mesfin Redi, for his genuine day to day follow up, advices, guidance, valuable suggestions, encouragement and continuous and devoted support during the entire period of this project and before that. It is intellectually stimulating and rewarding experience to work with him. I truly feel privileged in getting him as my advisor during my stay in Addis Ababa University.

I would like to thank Dr. Ahmed Mustefa for his genuine cooperation and guidance on computational work and in running the NMR spectrum of my compound.

I am grateful to Ato Tesfay Atsbeha and Taye Beyene for their cooperation in the computational techniques.

My special thanks go to Haromaya University /Department of Chemistry/ for their permission in using Spectrofluorometer and providing any necessary material while measuring fluorescence.

I also wish to acknowledge Mekelle University for their financial support during my stay in Addis Ababa University.

II. Table of content

Page

III. List of tables

Table **Pages**

IV. List of figures

V. Abstract

This paper mainly tends to explain the dual fluorescence of alizarin and its structures that are responsible for the existence of fluorescence emission spectra different from absorption spectra. And uses structural isomerism (Tautomersim) to explain the dual fluorescence shown by this compound. As well the affect of the $_{P}$H of the media, polarity of the solvent and hydrogen bond forming tendency of solvents on both absorption and fluorescence emission spectra of alizarin were studied. Four alizarin tautomers were selected for simplicity that may exist in both ground and excited state or only in one of the two states. The structure of this compound is investigated both experimentally and theoretically. Experimentally its absorption, fluorescence emission spectra and ^{1}H NMR were done and computational techniques (HF, DFT and semiemperical) were used to calculate total energy, Gibbs free energy, dipole moment, excitation energy and other molecular parameters.

Key words: Alizarin, fluorescence, tautomers, computational, absorption, protic, aprotic and ^{1}H NMR.

Tautomersim and Spectral Properties of Alizarin
(1,2-Dihydroxyanthracene-9, 10-dione)

1. Introduction

It has been found that the absorption spectra of some donor-acceptor type compounds are different from emission spectra [1]. Specially, compounds, which are bifunctional of oxygen with oxygen, nitrogen with nitrogen, oxygen with nitrogen or other related structures, show emission spectra that are different from the absorption spectra both in number of bands and shape of bands. The existence of two different absorption bands for a compound indicates the existence of two isomers of that compound in the ground state or more than one mode of transition. Similarly, the existence of two different emission bands is attributed to the existence of two isomers in the excited states or more than one mode of transitions [2, 3].

Alizarin has electronic structure similar to those groups of compounds, which have been proved to have the above-mentioned properties. Attributed to its tendency to exist in tautomeric forms it is expected to have unusual emission bands. This compound may exist in different tautomric forms in the ground and excited states [4]. Since ground and excited states differ in their electronic structures, different processes may occur in the two states. Alizarin, or 1,2-dihydroxyanthraquinone or mordant red, is the red dye originally derived from the root of the madder plant. In 1869, it became the first natural pigment to be duplicated synthetically. Madder has been cultivated as a dyestuff since antiquity in central Asia and Egypt, where it was grown as early as 1500 BC. Clothes were dried with this madder root pigment, because of its water solubility its application as a dye was limited. By 1804, the English dye maker George Field had developed a technique to *lake* madder by treating it with alum. This turned the water-soluble madder extract into a solid, insoluble pigment. This resulting madder lake had a longer-lasting color, and could be used more versatility, for example by blending it into paint. Alizarin can be combined with varying strengths of alkali or acids to create different hues of red. A strong alkali will create a violet-blue color, while a strong acid will create a yellowish red. The metallic salts used also influence the color of the lakes: the alumina lake is rose

red or bluish red with calcium, the tin lake is red-violet, the iron lake is black-violet, and the chrome lake is brown-violet or red-brown. Unlike other lakes, alizarin does not bleed red when boiled with sodium carbonate solution. Also, it does not dissolve when boiled with ammonia. This allows alizarin to be easily separated from other substances like carmine. Though alizarin is not highly toxic, it shouldn't be handled for long periods of time, eaten, or rubbed in eyes. It has been found to be related to cancer causing anthraquinones.

Alizarin has a number of applications besides for making dyes. Madder was formerly used in large quantities for dyeing textiles and is still the color for French military cloth. In a biochemical assay to determine, quantitatively by colorometery, the presence of calcific deposition by cells of an osteogenic lineage. Also used in spectrofluorimetric determination of boron in soil, detection of anions like fluoride and qualitative and quantitative determination of metal ions [5, 6]. In clinical practice it is also used to stain synovial fluid to assess for basic calcium phosphate crystals.

2. Literature Review

The direct conjugation of the donor acceptor molecular groups seems to be necessary for efficient electron transfer, particularly in the photon induced electron transfer reactions [7]. To be precise, after electron excitation, electron transfer takes place from initial molecular orbital (MO) of the donor (D) state, to a MO of the final of the acceptor (A) state. When the donor and acceptor groups are part of a single molecule, the resulting electron transfer is called intramolecular. If an electron transfer from D to A is energetically feasible in the excited state, the product of such intramolecular electron transfer reaction is charge-separated species, $D^{+}\cdots A^{-}$, and its electronic structure corresponds to the ground state of the free radical ion pair of opposite charges, consisting of the radical cation, D^{+} and radical anion, A^{-}. This results in changes in the dipole moment values between ground and excited state that led to the charge transfer (CT) state.

Relaxation processes e.g. pyramidazation or planarization, linearization, bending or twisting, and tautomerism etc, accompanied by during or after electron transfer

reactions, will cause various modifications in their electronic structure of the excited molecule. It is interesting to know which of the reaction coordinate determines the feasibility of electron transfer in these donor-acceptor systems and may lead to dual fluorescence [8,9].

In general, fluorescent compounds possess a single fluorescence band, there is however, where two fluorescence bands are observed for some donor-acceptor type compounds even in the case of simple donor acceptor benzene called 4-*N*, *N*-dimethylamino is preferentially stabilized by solvation. This has led to the nomenclature in photophysics: 'A' band for the "anomalous" emission from the ^1L_a-type state or charge transfer (CT) state and 'B' band for the normal short wavelength emission from the ^1L_b-type state or locally excited state (LE) state. These emitting states are also called B^* and A^* states, and can be in thermal equilibrium. Lippert et al. observed that the dual fluorescence strongly depends on the solvent polarity and on the temperature [8-10]. In polar solvents, the long wavelength fluorescence band grows in relative intensity, while the intensity of the first band decreases with increasing polarity of the medium. Though TICT model explains the unusual fluorescence spectra of some of these donor-acceptor compounds, it doesn't explain these properties for other types of compounds. Thus, other models were developed for these groups of compounds.

There have been various mechanisms proposed to explain the phenomena of dual fluorescence, and these are as follows:

(i) TICT- mechanism (Twisted Intramolecular Charge Transfer)

The TICT model was first put forward by Grabowski and coworkers to account for the observation that the dual fluorescence of DMABN with its "normal" band (B- band) and its "anomalous" one depends on the conformational freedom of the dimethylamino (DMA) group, coupled with an electron transfer in the orthogonal conformation. In the case of DMABN, there exists a reaction path in the excited state leading from the near planar conformation (emitter of the B-band) to an excited photochemical product with an energetic minimum at the perpendicular conformation (emitter of the A-band). These two emitting states possess a mother-daughter relationship, which has been revealed by direct

kinetic measurements. In many cases, the back reaction $A^* \rightarrow B^*$ also occurs leading to an excited state equilibrium. The ground state of DMABN is known to possess an energy barrier for the perpendicular conformation (the rotational barrier), therefore the emission from the perpendicular excited-state minimum occurs to a repulsive potential and is expected to lead to structure less spectra [11]. The key point here is that the reaction coordinate is not only the intramolecular twisting motion but involves other coordinates, too, such as electron transfer, solvent dipolar relaxation and, most probably, some rehybridization at the amino nitrogen. For the perpendicular TICT conformation, donor (dialkylamino group) and acceptor (benzonitrile) π-orbital are orthogonal (zero overlap) and thus decoupled leading to a maximum for the dipole moment in the excited state (and a minimum in the ground state) [7-10].

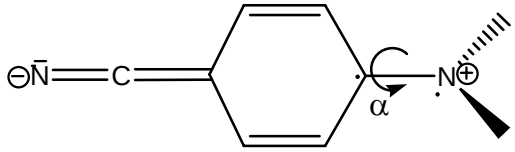


Fig. 1 Excited state rotation of amine group.

(ii) Pseudo – Jahn-Teller Mechanism

Zachariasse et al. found a new explanation for the occurrence of dual fluorescence in DMABN based on a Pseudo – Jahn-Teller (PJT) distortion of the molecular structure. It correlates between the efficiency of the CT state formation and the ^1L_a– ^1L_b energy gap in the absorption spectrum. They postulated that the proximity of these two electronic states favors the CT state. The PJT coupling of ^1L_a and ^1L_b states via the inversion mode (rehybridization) of the amino group is assumed to lead to a pyramidal geometry in the ICT state [9].

(iii) Rehybridization of the acceptor (RICT model)

Apart from the amino group (donor) involvement in the CT state, there can also be another site of structural changes in the cyano substituent (acceptor), that is, a bending of the cyano group (rehybridization) taking place in the excited state. It was suggested that the latter could be responsible for the anomalous emission from the A^* state [9,10].

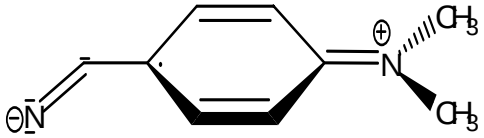


Fig.2 Excite state rehybridization of cyano group.

(iv)Tautomersim as a reason for Dual fluorescence

In addition to the above mechanisms that are responsible for the existence of emission spectra different from absorption spectra. Tautomerism is also responsible for the existence of different emission spectra for a compound [12]. Tautomerism causes a compound to have two different absorption or emission bands. Because tautomerism causes a compound to exist in two different equilibrium electronic states, i.e. two different spectra is expected for a compound. Different factors affect the existence of a compound in the tautomeric equilibrium, such as solvent polarity, solvent hydrogen bonding ability and the PH of the media [13-15]. Because solvent interacts with the sample, it stabilizes the sample differently in the excited and ground states existing in different electronic structure. These solvent effects enhance the tautomeric equilibrium in the excited state for some types of compounds and in the ground state for other types of compounds [16,17].

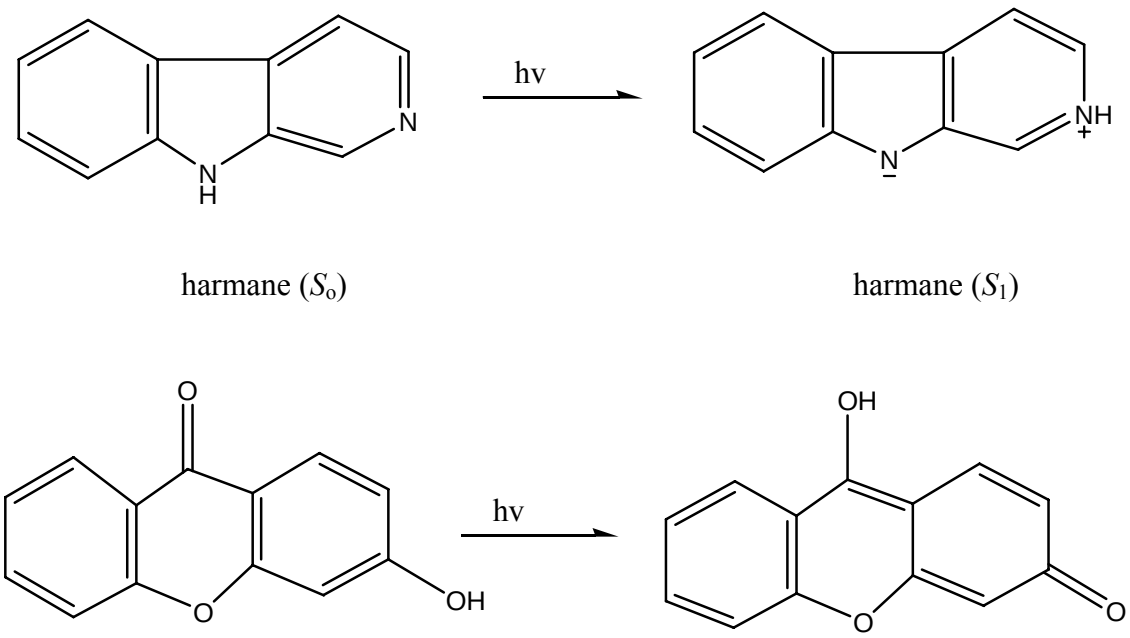
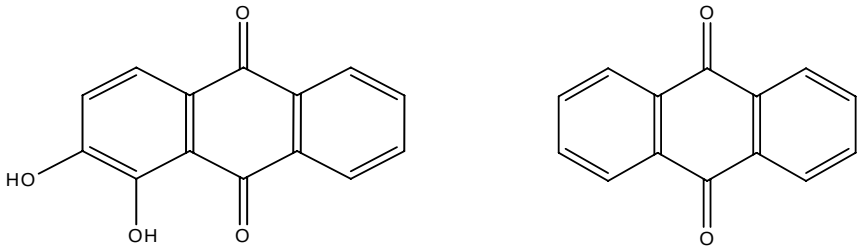


Fig. 3 Compounds that can undergo excited state proton transfer.

The spectral and photophysics of many compounds have been studied by using absorption and fluorescence spectroscopes. The long wavelength absorption of some of these compounds, for instance 3-hydroxy-9H-xanthen-9-one and 9-hydroxy-3H-xanthen-3-one show changes in the absorption and fluorescent spectra with a change in acidity and undergo isomerization in the excited state. This variation is attributed to the presence of two fluorescent ground state conformational or two structural isomers proportionally or the generation of the two conformational or two structural isomers in proportional amount in the excited states [2, 3].

The non-radiative deactivation process competes with the fluorescence emission of compounds that can fluoresce. In the past decades, an increasing number of researcher have attributed the non-radiative deactivation of compounds to the formation of the so called twisted intramolecular charge transfer (TICT) state from the S_1 single excited state.

The population probability of this TICT depends on the electron donor-acceptor capacities of the involved partners and on the solvent polarity, which would stabilize the highly polar structure [15,18,19]. The TICT hypothesis seems to describe well the behavior of simply substituted benzenes, pyrimidines, and probably pyridines. Doubts are arising when larger aromatic systems are involved [14]. Structural isomerism (Tautomersim) well explains the dual fluorescence of D-A type compounds like 3-hydroxy-9H-xanthen-9-one [20]. The candidate compound, 1,2-dihydroxyanthracene-9, 10-dione (Alizarin) derived from anthraquinone, has similar structure with the compounds mentioned above and expected to show excited and ground state intramolecular proton transfer (ESIPT).



1,2-dihydroxyanthracene-9, 10-dione (Alizarin) Anthracene-9, 10-dione

Fig.4 Anthracene and its family (Alizarin).

The compound is investigated experimentally by UV-Vis spectrophotometer and fluorometer and theoretically by computational techniques. First, brief discussions of the techniques are made followed by discussion of the result of experimental part and compression of the experimental result with theoretical result.

- **Visible and Ultraviolet Spectroscopy**

An obvious difference between certain compounds is their color. Organic compounds have different colors based on the length of conjugation and a particular functional group in it i.e. –OH, NH_2, -C=O, etc. Although we see sunlight (or white light) as uniform or homogeneous in color, it is actually composed of a broad range of radiation wavelengths in the ultraviolet (UV), visible and infrared (IR) portions of the spectrum. Colors of the visible portion can be separated by passing sunlight through a prism, which acts to bend the light in differing degrees according to wavelength.

Electromagnetic radiation is commonly treated as a wave phenomenon, characterized by a wavelength or frequency. Wavelength is defined as the distance between adjacent peaks (or troughs), and may be designated in meters, centimeters or nanometers (10^{-9} meters). Frequency is the number of wave cycles that travel past a fixed point per unit of time, and is usually given in cycles per second, or hertz (Hz). Visible wavelengths cover a range from approximately 400 to 800 nm and Ultraviolet approximately from 1nm-400nm. It is sub-divided in to near UV (NUV) (400-200) nm, far or vacuum UV (FUV or VUV) (200-10) nm and extreme or deep UV (EUV or DUV) (31-1) nm. Electronic structure of those compounds absorbing in the (NUV) and visible region are investigated. Those compounds absorbing in the VUV region requires more sophisticated instruments, which are costy, to use.

In organic chemistry common feature of all colored organic compounds is a system of extensively conjugated pi-electrons. Increasing the length of conjugation makes a compound absorb in the visible region of electromagnetic radiation and get a particular color depending on the length of conjugation and the functional group with in it.

2.1 UV-Visible Absorption Spectra

The interaction between electromagnetic radiation and matter attributed to dual nature of both created good opportunity to investigate compounds both qualitatively and quantitatively. EMR in the UV-Vis region enable one to analyze electronic structure of

compounds because the energy of EMR in this region is able to cause electronic promotion.

The energy of photon absorbed is related to the extent of conjugation and other functional groups of the compound. Commercial optical spectrometers enable such experiments to be conducted with ease, and usually survey both the near ultraviolet and visible portions of the spectrum. The visible region of the spectrum comprises photon energies of 36 to 72 kcal/mole, and the near ultraviolet region, out to 200 nm, extends this energy range to 143 kcal/mole. Ultraviolet radiation having wavelengths less than 200 nm is difficult to handle, and is seldom used as a routine tool for structural analysis. The energies noted above are sufficient to promote or excite a molecular electron to a higher energy orbital. Consequently, absorption spectroscopy carried out in this region is sometimes called "electronic spectroscopy". As a rule, energetically favored electron promotion will be from the highest occupied molecular orbital (HOMO) to the lowest unoccupied molecular orbital (LUMO), and the resulting species is called an excited state. UV-Vis absorption spectra (spectrum) are the graph of intensity of absorbed radiation versus the wavelength of radiation. Different molecules absorb radiation of different wavelengths. An absorption spectrum will show a number of absorption bands corresponding to structural groups within the molecule [21].

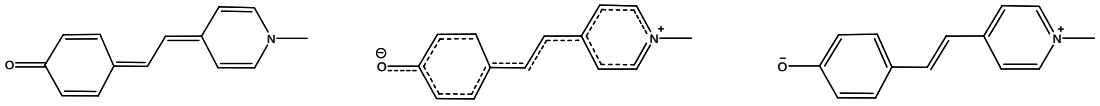
2.1.1 Factors Affecting UV-Vis Absorption Spectra

Different factors affect the absorption spectra of compounds. Intensities, position and shape of absorption bands are affected by temperature, solvent, $_{P}$H of the media. Under this heading the effect of solvent will be treated briefly.

2.1.2 Effect of Solvent

When absorption spectra are measured in solvents of different polarity, it is found that these solvents usually modify the position, intensities, and shapes of the absorption bands [21]. These changes are a result of physical intermolecular solute-solvent interaction forces (such as ion-dipole, dipole-dipole, dipole-induced dipole, hydrogen bonding, etc.),

which above all tend to alter the energy difference between ground and excited state of the absorbing species containing chromophore. The medium influence on absorption spectra can be considered by comparing the spectral changes observed (a) on going from the gas phase to solution, or (b) simply by changing the nature of the solvents. Because of the difficulty in measuring the absorption spectrum in the gas phase, the second method is used most of the times. The term solvatochromism is used to describe the pronounced changes in position (and sometimes intensity) of a UV-Vis absorption band that accompanies a change in the polarity of the medium. A hypsochromic (or blue) shift with increasing solvent polarity is usually called negative solvatochromism. The corresponding bathochromic (or red) shift is termed positive solvatochromism. Solvent affect absorption band by altering the energy difference between the ground and excited states of the absorbing species. UV-Vis spectrophotometer deals with electronic transition, the electronic structure of molecule in the ground state is different from the electronic structure of the excited state. Thus, in most cases the dipole moment of the molecule in the ground state is different from that of the excited state. If the dipole moment of the molecule in the ground state is greater than that of the excited state, polar solvents stabilize the molecule in the ground state than they do in the excited state. Since the energy of the molecule in the ground state is lowered more than its energy in the excited state, the energy gap is increased. As a result the absorption band shifts to lower wavelength region, hypsochromic (or blue) shift. However, if the dipole moment of the excited state is greater than that of the ground state, more polar solvents stabilize the excited state more. And consequently, the energy gap is lowered (bathochromic) shift is observed. Alteration of energy gap of the excited and ground state of absorbing species with polarity of solvents is one of the ways by which solvent effect on absorption band is described. Solvents also affect absorption spectra because they may alter the electronic structure of the molecule in the ground state, i.e. electronic structure of some molecules in different solvents is different. Especially those molecules, which are able to exist in different resonance structures, exhibit this character. Interesting compound exhibiting this behavior is 1-methyl-4 [(4-oxocyclohexadienylidene]-1,4-dihydropyridine also called Brooker`s merocyanine [21].



Quinonoid structure (C_6H_{12}), λ_{max} = 592 nm

Cyanine Structure (CHCl3), λ_{max} = 620nm

Benzenoid structure (H2O), λ_{max} = 442 nm

Fig. 5 Solvent effect on absorption spectra of 4-(2-(1-methylpyridin-4 (1H)-ylidene)ethylidene)cyclohexa-2,5-dienone and its resonance isomer.

In non-polar solvents the quinonoid structure [2] is the dominant species, in less polar solvents the intermediate structure is the dominant species with its characteristic absorption and in polar solvents the benzenoid structure is dominant showing its characteristic absorption band. Therefore, solvents affect the absorption bands of compounds by changing the electronic structure of the compound in the ground state. With changing the polarity of solvents the electronic structure of compounds gets changed and consequently the absorption bands are changed. Changing solvent polarity also causes a dramatic change in molecular geometry. This can be shown by calculating the changes in bond length and net π-electron charges with varying solvent polarity.

2.2 Fluorescence Spectrometry

Generation of luminescence through excitation of a molecule by ultraviolet or visible light photons is a phenomenon termed photoluminescence, which is formally divided into two categories, fluorescence and phosphorescence, depending upon the electronic configuration of the excited state and the emission pathway [23].

Fluorescence is a luminescence, in which absorption of a photon of radiation by molecular causes the emission of photon at a longer wavelength. The energy difference between the absorbed and emitted photon ends up as molecular vibrations or heat or other excited state processes. Attributed to the energy loss in the excited state by the processes mentioned above the absorbed and emitted photon by the molecule are in different region of electromagnetic radiation. The energy gap between the two processes depends on the nature of the fluorophore, temperature, solvent, pH of the media, etc. Fluorescence is

named after the mineral fluorite, composed of calcium fluoride, which often exhibits this phenomenon.

At room temperature most molecules occupy the lowest vibrational level of the ground electronic state, and on absorption of light they are elevated to produce excited states. The simplified diagram below shows absorption by molecules to produce either the first, S_1, or second S_2, excited state.

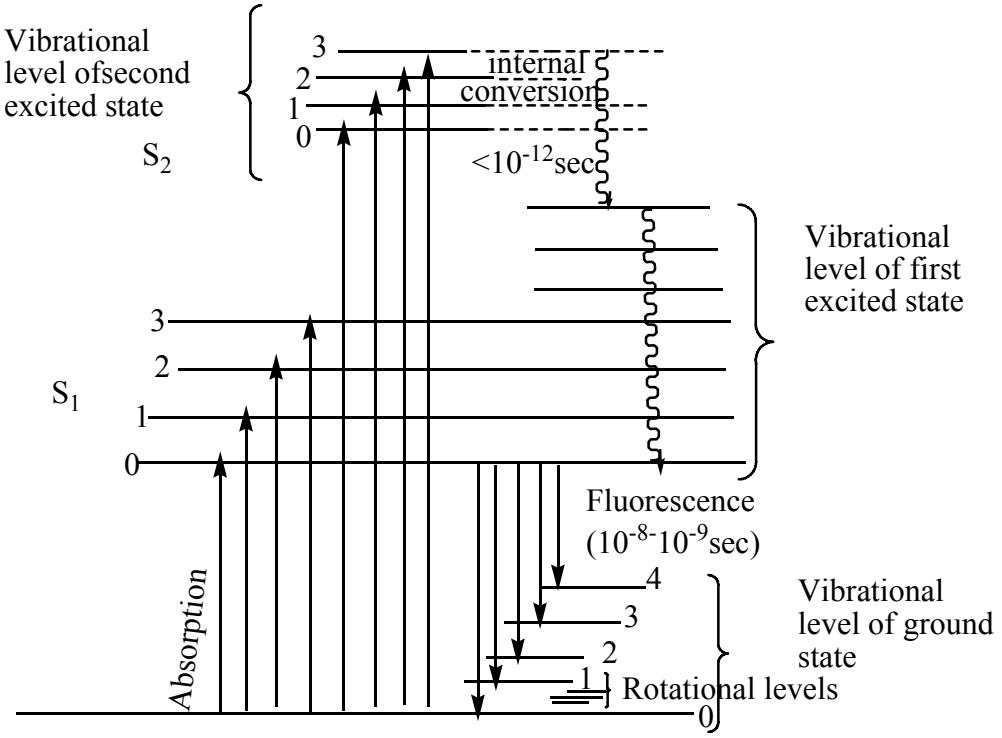


Fig. 6 Schematic diagram of Transitions giving rise to absorption and fluorescence emission spectra.

Excitation can result in the molecule reaching any of the vibrational sub-levels associated with each electronic state. Since the energy is absorbed as discrete quanta, this should result in a series of distinct absorption bands. However, the simple diagram above neglects the rotational levels associated with each vibrational level and which normally increase the number of possible absorption bands to such an extent that it becomes impossible to resolve individual transitions. Two basic types of spectra can be produced by a fluorescence spectrometer [2,14]. In a fluorescence spectrum, or emission spectrum, the wavelength of the exciting radiation is held constant (at a wavelength at which the analyte absorbs) and the spectral distribution of the emitted radiation is measured. In an

excitation spectrum, the fluorescence signal, at a fixed emission wavelength, is measured as the wavelength of the exciting radiation is varied. Because an analyte can fluoresce only after it has absorbed radiation, an excitation spectrum identifies the wavelengths of light that the analyte is able to absorb. Thus, subject to certain constraints, the excitation spectrum of a molecule should be the same as its UV-Vis absorption spectrum. The excitation spectrum for a compound should not change as the emission wavelength is varied. Whenever the excitation spectrum varies with choice of emission wavelength, there is good reason to believe that two or more different substances are responsible for the observed fluorescence. Fluorescence occurs when a molecule excited by absorption of radiation relaxes to its ground state.

$$\begin{aligned} &\textit{Excitation}: \quad S_0 + h\nu \rightarrow S_1 \\ &\textit{Emission}: \quad S_1 \rightarrow S_0 + h\nu' \end{aligned}$$

where $h\nu$ is photon energy. State S_0 is called the ground state of the fluorophore (fluorescent molecule) and S_1 is its first (electronically) excited state. The specific frequencies of exciting and emitted light are dependent on the particular system. A molecule in its excited state, S_1, can relax by various competing pathways. It can undergo 'non-radiative relaxation' in which the excitation energy is dissipated as heat (vibrations) to the solvent. Excited organic molecules can also relax via conversion to a triplet state which may subsequently relax via phosphorescence or by a secondary non-radiative relaxation step. Relaxation of an S_1 state can also occur through interaction with a second molecule through fluorescence quenching. Molecules that are excited through light absorption or via a different process (e.g. as the product of a reaction) can transfer energy to a second 'sensitizer' molecule, which is converted to its excited state and can then fluoresce [24,25].

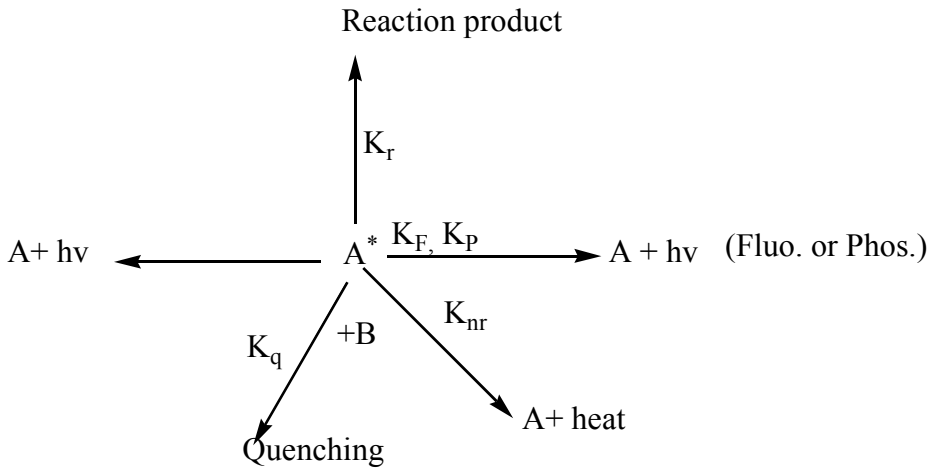


Fig. 7 Schematic diagram of various relaxation pathways of excited molecule.

2.2.1 Factors Affecting Fluorescence Spectra

A variety of environmental factors affect fluorescence emission, including interactions between the fluorophore and surrounding solvent molecules (dictated by solvent polarity), other dissolved inorganic and organic compounds, temperature, pH, and the localized concentration of the fluorescent species [2,14,26]. The effects of these parameters vary widely from one fluorophore to another, but the absorption and emission spectra, as well as quantum yields, can be heavily influenced by environmental variables. In fact, the high degree of sensitivity in fluorescence is primarily due to interactions that occur in the local environment during the excited state lifetime.

2.2.2 Solvent Effect

Like UV-Vis absorption spectra fluorescence spectra is also affected by polarity of solvents. Once the excited state of a molecule is formed in solution, the excited state molecule interacts to varying degrees with surrounding solvent molecules, depending on their polarity, before returning to the ground state. These excited-state solute-solvent interactions of fluorescent molecules are often reflected in the spectral position and shape of the emission bands as well as in the lifetimes of the excited state molecules [21].

Solvent dependence of fluorescence emission position is sometimes called solvatofluorochromism. Fluorescence usually occurs from the S_1 state to the S_0 electronic state by emission of photon. The $0 \rightarrow 0$ transition is generally the same for absorption and emission. According to stoke`s rule, the fluorescence maximum is always located at lower wavenumbers (higher wavelength) than absorption maximum because of the loss in energy due to vibrational relaxation. The gap between the maximum of the first absorption band and the maximum of the corresponding fluorescence band is called stokes shift, and is usually expressed in wavenumbers as $\Delta\tilde{\nu} = \nu_a - \nu_f$. This stokes shift provides valuable information on the excited state. When the dipole moment of a fluorescent molecule is larger in the excited state than in the ground state (i.e. $\mu_e > \mu_g$), the differential solvation of the two states by solvents of varying polarity gives rise to an increase in stokes shift with increasing solvent polarity. When considering the solvent dependence of the position of emission bands, the finite relaxation time, τ_R, for the rearrangement of the solvent molecules surrounding the solute molecule in the Franck-Condon excited state and the finite lifetime, of the molecule in the excited state need to be considered. In the case of $\tau_R > \tau_e$, the emission will occur before any arrangement of solvent molecules in the solvation shell takes place. The initial state of emission process is the Franck-Condon excited state and the final state is the equilibrium ground state. Hence, the wave number of emission will be equal to the wave number of corresponding absorption. In the case of $\tau_R \square \tau_e$, reorientation of the solvent molecules can take place after electronic excitation and a relaxed excited state is obtained in which another solvation equilibrium has been established. It is from this equilibrium state that fluorescence occurs at room temperature.

After the fluorophore has been excited to higher vibrational levels of the first excited singlet state S_1, excess vibrational energy is rapidly lost to surrounding solvent molecules as the fluorophore slowly relaxes to the lowest vibrational energy level (occurring in the Pico second time scale). Solvent molecules assist in stabilizing and further lowering the energy level of the excited state by re-orienting (termed solvent relaxation) around the excited fluorophore in a slower process that requires between 10 and 100 Pico seconds.

This has the effect of reducing the energy separation between the ground and excited states, which results in a red shift (to longer wavelengths) of the fluorescence emission. Increasing the solvent polarity produces a correspondingly larger reduction in the energy level of the excited state, while decreasing the solvent polarity reduces the solvent effect on the excited state energy level. The polarity of the fluorophore also determines the sensitivity of the excited state to solvent effects. Polar and charged fluorophores exhibit a far stronger effect than non-polar fluorophores [21].

A general explanation of solvent effects on fluorescence spectra is based on the differential solvation of the fluorescent molecules (also called fluorophores) in their ground and excited states, mediated by various non-specific and specific intermolecular forces acting between the solute and solvent. The strength of such a force depends up on the similarity in the polarity of the solvent and the fluorophore.

2.2.3 Temperature Effects

Changes in temperature affect the viscosity of the medium and hence the number of collisions of the molecules of the fluorophore with solvent molecules and the strength of hydrogen bonding. Fluorescence intensity is sensitive to such changes and the fluorescence of certain fluorophores shows temperature dependence. In such cases the use of thermostatted cell holders is to be recommended. Normally, it is sufficient to work at room temperature with the provision that any sample procedure involving heating or cooling must also allow sufficient time for the final solution to reach ambient before measurement [26,27].

2.2.4 pH Effects

Relatively small changes in pH will sometimes radically affect the intensity and spectral characteristics of fluorescence. Accurate pH control is essential and, when particular buffer solutions are recommended in an assay procedure, they should not be changed without investigation. Most phenols are fluorescent in neutral or acidic media, but the presence of a base leads to the formation of nonfluorescent phenate ions. 5-

hydoxyindoles, for example, show a shift in fluorescence emission maximum from 330 nm at neutral pH to 550 nm in strong acid without any change in the absorption spectrum [14,16].

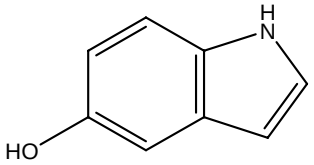


Fig. 8 5-hydoxyindole.

2.2.5 Concentration (Inner-Filter) Effects

Fluorescence intensity will be reduced by the presence of any compound that is capable of absorbing a portion of either the excitation or emission energy [24]. At high concentrations this can be caused by absorption due to the fluorophore itself. More commonly, particularly when working with tissue or urine extracts, it is the presence of relatively large quantities of other absorbing species that is troublesome. The purpose of extraction procedures is usually to eliminate such species so that the final measurement is made upon a solution essentially similar to the standard. At low concentration the emission of light is uniform from the front to the back of sample cuvette. At high concentration more light is emitted from the front than the back. Since emitted light only from the middle of the cuvette is detected the concentration must be low to assure accurate Φ_F measurements.

2.2.6 Quenching

Decrease of fluorescence intensity by interaction of the excited state of the fluorophore with its surroundings is known as quenching [24]. Quenching is any process in which a sample constituent decreases the fluorescence quantum yield for the analyte. Among the most common fluorescence quenchers is O_2; removal of oxygen from a sample before fluorometric analysis is often advisable. One-way that fluorescence quenching can occur is intermolecular electronic energy transfer:

$$A^* + B \rightarrow A + B^*$$

Here an excited analyte molecule (*A**) transfers excitation energy to a quencher molecule *B*, causing de-excitation of *A* and forming an excited quencher molecule, *B**. If *B** is a fluorescent species, its fluorescence (called sensitized fluorescence) may then be observed. This phenomenon can allow one to observe fluorescence from a molecule (*B*) that may be difficult to excite directly. More often, however, these processes are a nuisance. Not only do they cause a decrease in the fluorescence signal observed for a given concentration of analyte (*A*) in the sample, but also they may produce unwanted background fluorescence signals; that is, *B* may act both as a multiplicative and an additive interference [11].

2.2.7 Adsorption, Photodecomposition and Oxidation

Loss of organic substances by adsorption onto the walls of the container becomes particularly troublesome at the sub-microgram level. New glass surfaces should be thoroughly cleaned in acid before use and measurements of aromatic substances in non-polar solvents should be avoided. Often the addition of a small quantity of a polar solvent to a non-polar one will greatly reduce adsorption losses. Fluorimeters employ intense light sources to produce high sensitivity and in some cases the level of incident light may be sufficient to decompose the sample under investigation.

3. Computational Method of Molecular Treatment

Earlier classical methods were used to describe the properties of microscopic systems theoretically. Nowadays wave mechanics is getting more important in describing the properties of microscopic systems and in predicting the structure of molecules [4,10]. Some of the properties of molecules that can be predicted by this technique are structure (i.e. the expected positions of the constituent atoms), absolute and relative (interaction) energies, electronic charge distribution, dipoles and higher multipole moments, vibrational frequencies, reactivity or other spectroscopic quantities. In order to describe the properties of the microscopic systems, wave mechanics uses a postulate, which states for any system there exists a wave function, Ψ, and operator corresponding to each property of the system that act up on Ψ to return the observable properties of the system. For example, the operator that acts upon the wave function of the system to generate the energy of the system is Hamiltonian operator. Schrödinger equation is the basis for the quantum mechanical calculation of properties of microscopic systems. Schrödinger equation in time dependent or time-independent form is not solved except for very small systems. In order to solve Schrödinger equation for many electron systems a number of approximation methods, which differ in their accuracy, have been developed. Density functional theory (DFT), Hartree-Fock and semi-empirical approximation methods are largely used in computational chemistry to calculate and predict molecular properties [8,26].

3.1 Hartree-Fock Methods

For many electron systems Schrödinger equation is,

$$\frac{-\hbar^2}{2m}\frac{\partial^2\Psi(x,t)}{\partial x^2}+U(x)\Psi(x,t)=i\hbar\frac{\partial\Psi(x,t)}{\partial t}$$

is very difficult to solve. Hartree simplified the problem by making an assumption about the form of the many-electron wave functions namely the product of a set of single-

electron wave functions. In a uniform system these wave functions would take the form of simple plane waves. After simplifying the Schroedinger equation by the above approximation method, variation method can be used. Variational principles states that if a system is described by a set of unknown parameters the set of parameter values, which correctly describes the ground state of the system, is just that set of values which minimizes the total energy. The Hartree wave function is symmetric with respect to the exchange of the electrons. Therefore, the Hartree approach neglected the Pauli exclusion principle, which states that it is not possible for two electrons to exist at the same point in space with the same set of quantum numbers. Fock modified the Hartree wave function to overcome the problem. Fock extended the Hartree's procedure to Slater determinantal wave functions in order to meet the anti-symmetric nature of electrons. In the Hartree-Fock approach, many-electron wavefunction is specially constructed out of single-electron wave functions in such a way as to be antisymmetric. The Slater type orbitals are written as follows for N-electron system.

$$\Psi = \frac{1}{\sqrt{N!}} \begin{vmatrix} \Psi_1(1) & \Psi_2(1) \cdots \Psi_N(1) \\ \Psi_1(2) & \Psi_2(2) \cdots \Psi_N(2) \\ \vdots & \vdots & \vdots \\ \Psi_1(N) & \Psi_2(N) \cdots \Psi_N(N) \end{vmatrix}$$

3.2 Semi-Empirical Methods

Semi empirical Methods are based on Hartree-Fock theory using empirical, but make use of data derived from experiment and make many approximations in order to improve performance. Semi-empirical calculations are much faster than their *ab initio* counterparts. Their results, however, can be very wrong if the molecule being computed

is not similar enough to the molecules in the database used to parameterize the method [28, 29].

These methods are usually referred to through acronyms encoding some of the underlying theoretical assumptions. The most frequently used methods (MNDO, AM1, PM3) are all based on the Neglect of Differential Diatomic Overlap (NDDO) integral approximation, while older methods use simpler integral schemes such as CNDO and INDO. All three approaches belong to the class of zero differential overlap (ZDO) methods, in which all two-electron integrals involving two-center charge distributions are neglected. A number of additional approximations are made to speed up calculations (see below) and a number of parameterized corrections are made in order to correct for the approximate quantum mechanical model. How the parameterization is performed characterizes the particular semi empirical method. For MNDO, AM1, and PM3 the parameterization is performed such that the calculated energies are expressed as heats of formations instead of total energies. Methods, Zerner's Intermediate Neglect of Differential Overlap ZINDO and symmetric Intermediate Neglect of Differential Overlap SINDO are used to calculate excited states and hence predict electronic spectra [8].

Table. 1 Ground and excited state calculation methods used in semiemperical.

Acronym	**Full name**	**Underlying approximation**	**Parameters**	**Fitted-Parameters**
CNDO	Complete Neglect of Differential Overlap	CNDO	-	-
INDO	Intermediate Neglect of Differential Overlap	INDO	-	-
MINDO/3	Modified Intermediate Neglect of Differential Overlap, version 3	INDO	10	2
MNDO	Modified Neglect of Differential Overlap	NDDO	10	5
AM1	Austin Model 1	NDDO	13	8
PM3	Parametric Model number 3	NDDO	13	13

3.3 Density Functional Theory (DFT)

DFT is a second type of ab initio method, even though many of the most common functionals use parameters derived from empirical data, or from more complex calculations. In DFT, the total energy is expressed in terms of the total elctron density rather than the wave function. In this type of calculation, there is an approximate Hamiltonian and approximate expression for the total electron density. The main objective if density functional theory is to replace the many-body electronic wave function with electronic density as the basic quantity. Where as the many-body wavefunction is dependent on 3N variables, three spatial variables for each of the N-electrons, the density is only a function of three variables and is a simpler quantity to deal with both conceptually and practically [8,9, 16].

This paper mainly tends to explain the dual fluorescence of alizarin and its structures that is responsible for the existence of fluorescence emission spectra different from absorption spectra. And uses structural isomerism (Tautomersim) to explain the dual fluorescence as shown by this compound. As well the affect of the $_{P}$H of the media, polarity of the solvent and hydrogen bond forming tendency of solvents on both absorption and fluorescence emission spectra of alizarin are investigated. Four alizarin tautomers are selected for simplicity that may exist in both ground and excited state or only in one of the two states. The structure of this compound is investigated both experimentally and theoretically. Experimentally its absorption, fluorescence emission and NMR spectra were done and computational techniques (HF, DFT and semiemperical) were used to calculate total energy, Gibbs free energy, dipole moment, excitation energy and other molecular parameters.

4. Objective, Materials, procedures and methods

4.1 Objectives

a. General objective:

To investigate photophysical properties of tautomeric compounds existing in equilibrium

b. Specific objectives:

The specific objectives of this study include investigation and

- Determination of fluorescence emission spectra of alizarin.
- Comparison of absorption spectra and fluorescence emission spectra.
- Determination of effect of hydrogen bonding properties of solvents on the absorption and fluorescent emission spectra.
- Identification of structural features responsible for the dual fluorescence.
- Computation of important physical properties of Alizarin.

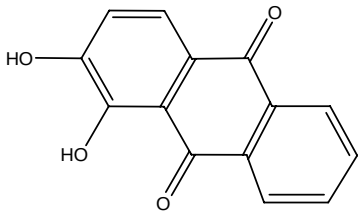
4.2 Experimental Part

About ten donor-acceptor type compounds were taken from store and UV-Vis spectrophotometer (SpectroPhotoGenesis 2PC) was used to screen three of them that have good absorption in the UV-Vis region. First, all the compounds were dissolved in four solvents (cyclohexane, dichloromethane, 1,4-dioxane and ethanol) and their absorbance were measured under almost the same experimental conditions. Alizarin, dithiazone and diphenyl thiocarbazone were found to have good absorption in this region in four of the solvents used. In running UV-Vis for these compounds quartz cuvett were used as sample holder and the spectrometer contains deuterium and tungsten lamp as light source for different regions. Fluorescence emission spectra were recorded on Spectrofluorometer (SHIMADZU CORPORATION ANALYTICAL, RF-5301PC) in

Haromaya University. Xenon Lamp was used as light source and quartz cuvette was used as sample holder in making our measurements. Emission spectra were recorded for the three compounds selected in all of the four solvents mentioned above. And only alizarin was found to fluoresce in the solvent we used and emission spectra were measured at many fixed excitation wavelengths. Under the same experimental conditions, excitation spectra were also recorded for this compound at different emission wavelength in three of the solvents used. To see the effect of $_P$H, H_2SO_4 and KOH were added to solution of alizarin in cyclohexane and ethanol in different beakers followed by measurement of fluorescence emission spectra. Concentration effect was also detected by dissolving varying amounts of alizarin in ethanol and 1,4-dioxane and then by measuring fluorescence emission spectra for different concentrations in both solvents. ^{1}H NMR of this compound in CCl_3D and DMSO were recorded by Bruker Avance 400 MHz Spectromter.

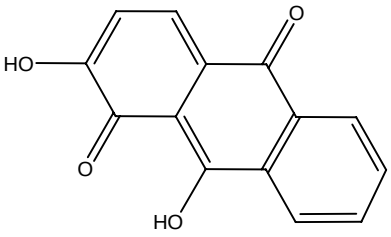
4.3 Computational Method

Quantum Chemical calculations were performed at the HF and DFT/B3LYP levels of theory with the Gaussian 03 package.13. Geometries were optimized at HF and DFT levels with the 6-31G* basis set with no symmetry constraints. The GIAO (gauge independent atomic orbital) calculation for^{1}H chemical shifts were performed at the DFT/B3LYP level with 6-311+G* basis set. The chemical shifts are referred to TMS for ^{1}H NMR (computational level for TMS compound was the same as for the tautomeric species). TDSCF and ZINDO were also used to calculate the excitation energies of the four tautomers. Based on the molecular structure of alizarin four tautomers can be proposed for which some molecular properties were calculated.



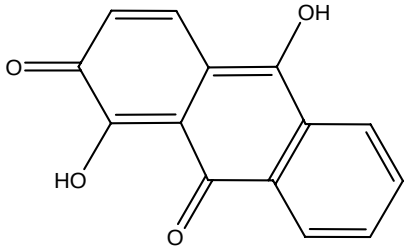
1,2-dihydroxyanthracene-9,10-dione

(Al-1)



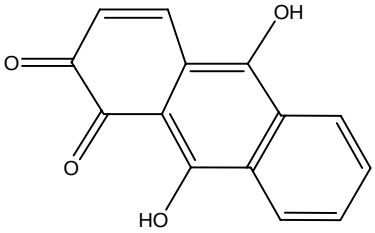
2,9-dihydroxyanthracene-1,10-dione

(Al-3)



1,10-dihydroxyanthracene-2,9-dione

(Al-2)



9,10-dihydroxyanthracene-1,2-dione

(Al-4)

Fig.9 Alizarin and its tautomers

5. Results and Discussion

5.1 Experimental Part

5.1.1 Absorption and Excitation Spectra of Alizarin

UV-Vis absorption spectrophotometer and spectrofluorometer were used to study or identify the absorption and emission position and the influence of polarity and hydrogen bonding properties of solvents on the position of bands, intensity and shape of the spectra as a whole. The absorption and excitation spectra of alizarin were recorded with solvents of different polarity (Cyclohexane, Dichloromethane, Dioxane and Ethanol). In the absorption spectra only a slight shift in the wavelength of maximum absorption (λ_{max}) was observed, indicating non-significant modification of the HOMO and LUMO energy states of the molecule through reaction field effects (Fig.1). Fig.1 shows with increasing polarity of solvents, the absorption band shows very small red shifting with polarity that may indicate the polarity of the molecule in excited state is greater than its polarity in the ground state.

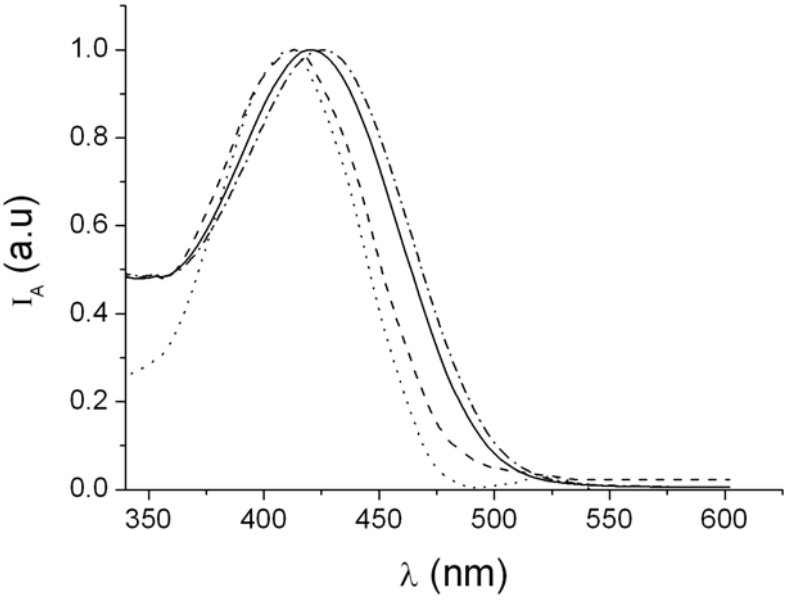


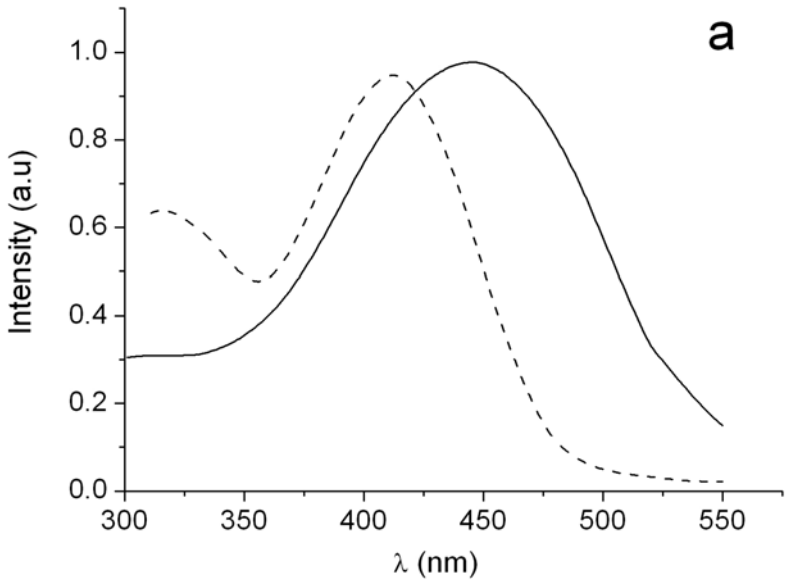
Fig.1. Normalized absorption spectra of Alizarin in 1,4-dioxane (—), cyclohexane (---), dichloromethane (···) and ethanol (-··-).

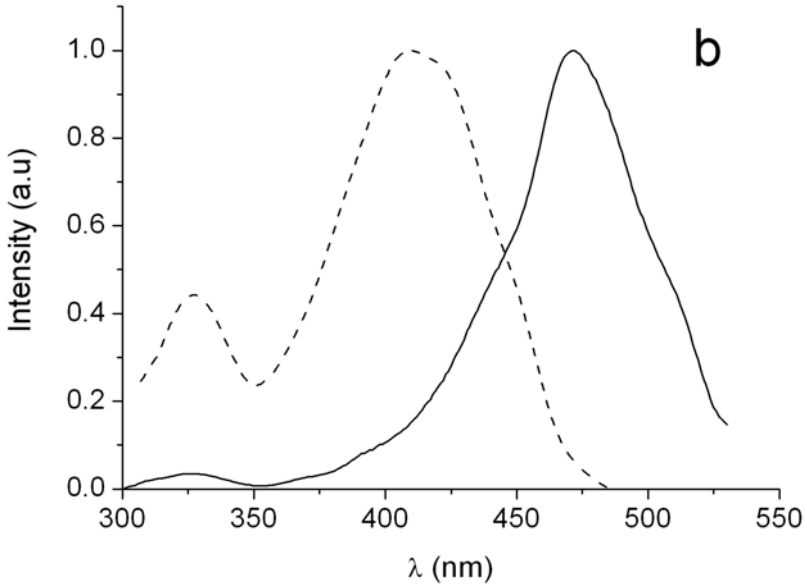
Absorption and excitation spectra of alizarin in different solvent show similarity in their band shape. As shown in Figure 2, in all solvents their band shape is similar but there is a difference in the position of the long wavelength bands between the excitation and absorption spectra. The short wavelength excitation and absorption bands are almost similar i.e. almost the shift is insignificant. Implying that the electronic structure responsible for this transition is not sensitive to the various possible types of interactions. The shift is pronounced between the long wavelength absorption and excitation bands in all of the solvents used. As sited in the theoretical section, alizarin is a compound that can exist in many tautomeric forms. In the absorption spectrum, the bands corresponding to the various species may not be resolved when the electronic structure of the species excited are closer since tautomers have similar physical properties. Consequently, broadened band is observed. Often most of the excited species may not exhibit fluorescence, and only some of them under go fluorescence. Since in the excitation spectrum (fluorescence intensity at fixed emission wavelength over range of absorption wavelengths) only the bands corresponding to the fluorescent molecule are observed, the difference between the two (absorption and excitation) is expected. Alizarin contains

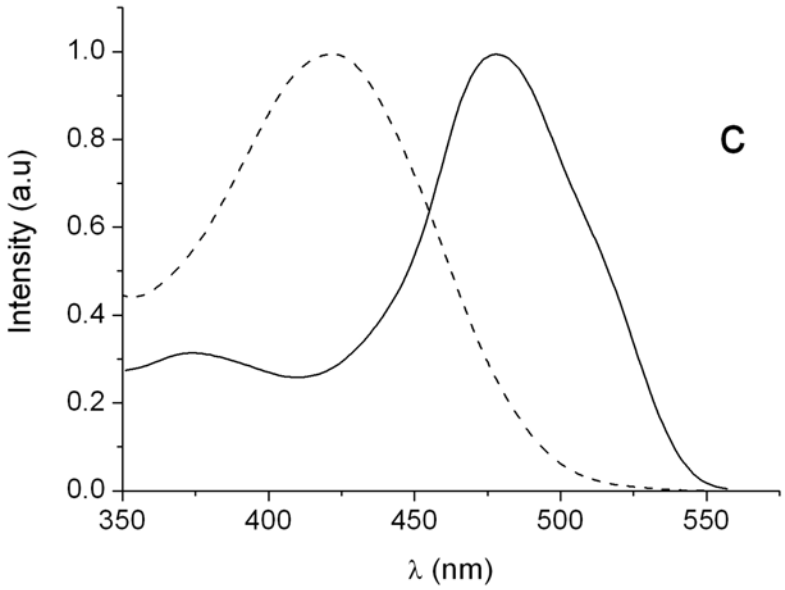
groups that can interact via various intermolecular and intramolecular forces; in absorption spectrum the influence of all these interactions may not be resolved as in the excitation spectrum.

For a single transition the band shape of absorption and excitation spectra are similar, and any pronounced variation, in the band shape, indicates the existence of more than one transion, which may be attributed to the existence of multiple absorbing species. Alizarin can tautomerize to give more than two tautomeric species and one may expect differences in absorption and excitation spectra band forms.

In the excitation spectrum shoulders are observed in addition to the bands indicating the possibility of formation of various hydrogen bonding complexes. This is more pronounced in the hydrogen bonding solvent, ethanol (Fig. 2d). The third excitation band is clearly seen in this solvent that may be due to one of the tautomers of alizarin or the formation of stable hydrogen bonding complex that can fluoresce.




b
Intensity (a.u)
1.0
0.8
0.6
0.4
0.2
0.0
300
350
400
450
500
550
λ (nm)


c
Intensity (a.u)
1.0
0.8
0.6
0.4
0.2
0.0
350
400
450
500
550
λ (nm)

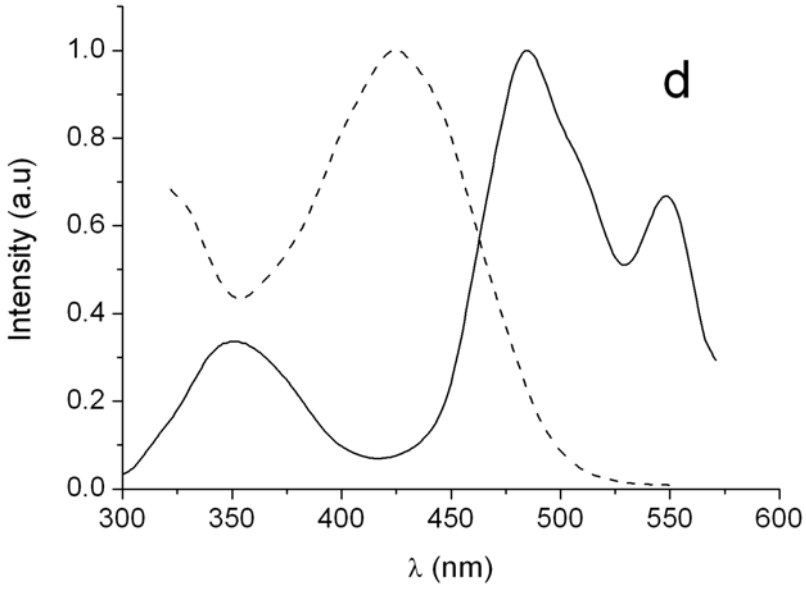


Fig. 2. Absorption (- - -) and excitation (—) spectra of alizarin in cyclohexane (a), dichloromethane (b), 1,4- dioxane (c) and ethanol (d).

5.1.2 Fluorescence Emission

The fluorescence spectra of this compound are not as simple as its absorption spectra. The influence of polarity, hydrogen-bonding properties of solvents and pH of the media on fluorescence emission spectra were dealt with although explanation of what is observed is more complex. In the emission spectrum of this compound two resolved bands are seen (Fig. 3). The shift in the position of these bands with polarity of solvent is insignificant (Fig. 3) indicating that the effect of solvent polarity on the excited state electronic structure is less. But the relative intensity of the two bands varies with solvent hydrogen bonding property and polarity. With increasing solvent polarity the intensity of the log wavelength emission band increases indicating that the electronic structure responsible for this band is favored in polar solvents. Even in hydrogen-bond donating solvents, the long wavelength emission band predominates. Thus, the relative intensity of the short wavelength emission band predominates in the non-polar solvent, cyclohexane, and the longer one predominates in hydrogen bonding solvent ethanol (Fig. 3).

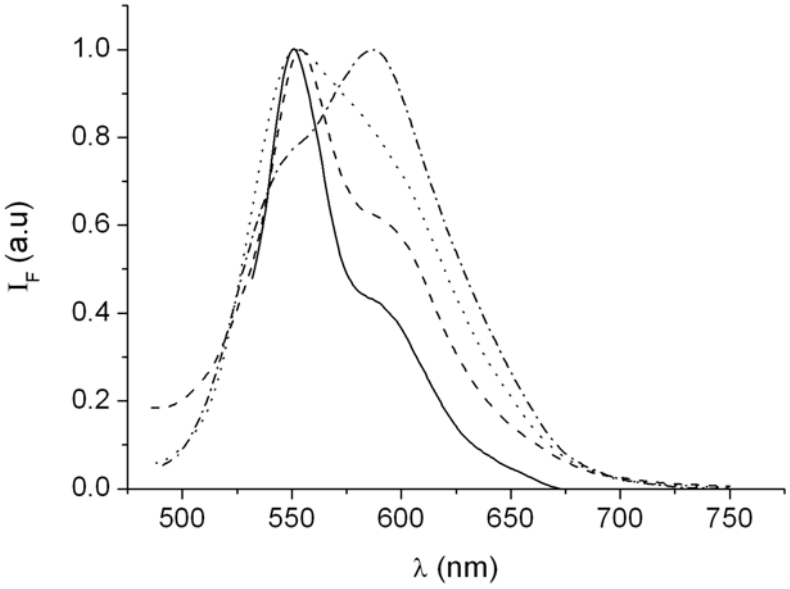


Fig. 3 Influence of polarity of solvents on fluorescence emission spectra of Alizarin at $\lambda_{ex} = 470$ nm in cyclohexane (—), dichloromethane (‾‾), 1,4-dioxane (···) and ethanol (-·-·-).

In aprotic solvents the compound showed two fluorescence emission bands at about 554 – 556 nm and 590 – 598 nm. In these solvents intensity of the long wavelength band is greater than the intensity of the short wavelength band whenever excitation wavelength is fixed in the range 400 – 430 nm with the increase in the intensity of both bands (Fig. 4 & 5). The short wavelength emission band relatively more intensified in cyclohexane than the other more polar solvents (Fig. 4). With increasing the excitation wavelength up to 480 nm the intensity of the short wavelength emission band is greater than that of the longer one (Fig. 4 & 5). The position of these bands does not shift significantly with polarity in aprotic solvents. One difference that is observed is that in the more polar solvent 1,4-dioxane the long wavelength fluorescence emission band is intensified and broadened (Fig.6) which may be attributed to the appearing of two unresolved band or influence of hydrogen bond interaction or the greater proportion of electronic structure responsible for this band in this solvent. In three of the aprotic solvents the short wavelength emission band is intensified relative to the longer one.

The two-resolved emission bands in the aprotic solvents vary only slightly, appearing at about 550 nm and 596 nm in cyclohexane, 554 nm and 598 nm in dichloromethane, at 556 nm and 595 nm in 1,4-dioxane (Fig. 4, 5 & 6). The longer and shorter fluorescence emission bands attain their maximum intensity at different excitation wavelength (Fig. 4, 5 and 6) indicating that different electronic structures are responsible for the observed emission bands [14,27]. The isoemission point observed in all solvent in the fluorescence emission spectra also supports the existence of two electronic structures at equilibrium. The isoemission points in aprotic solvents appear at i.e. at 577 nm in cyclohexane at 586 nm in dichloromethane and at 588 nm in 1,4-dioxane (Fig. 4, 5 &8).

For most of compounds only a single fluorescence band is observed. But it was found that some compounds show more than one fluorescence bands. For these types of compounds, different models were developed to explain this unusual property [30,31]. The types models that exactly explain the observed properties vary from compound to compound. The dual fluorescence of alizarin (Fig. 4, 5 & 6) may be assigned to the presence in the ground state of two electronically different absorbing species or the different types of processes taking place in the excited state leading to the generation of electronically different chemical species [2,14]. Conformational isomers or structural

isomers (tautomers) generation in the ground and excited states or in only one of the states is proposed for most of the compounds showing this unusual phenomenon [16,17]. Formation of conformation isomers in excited states by rotation a group or groups were found to explain well this phenomenon for compounds like DMABN. For compounds containing mobile protons this phenomenon is attributed to the formation of tautomer in the ground state or photon-induced proton transfer as described elsewhere in the background part. If only single excitation wavelength is responsible for the observed emission bands i.e. if emission spectrum is independent of excitation wavelength, excited state introversion (equilibrium) is responsible for the observed bands [30,33,34]. But if the emission spectrum shows dependence on excitation wavelength, equilibrium between ground state electronic structures is responsible for the observed bands. Therefore, one can easily identify whether equilibrium between ground state electronic structures or equilibrium between excited state electronic structures are responsible for dual fluorescence by checking the dependence of emission spectra on excitation wavelength and dependence of emission spectra on excitation wavelength.

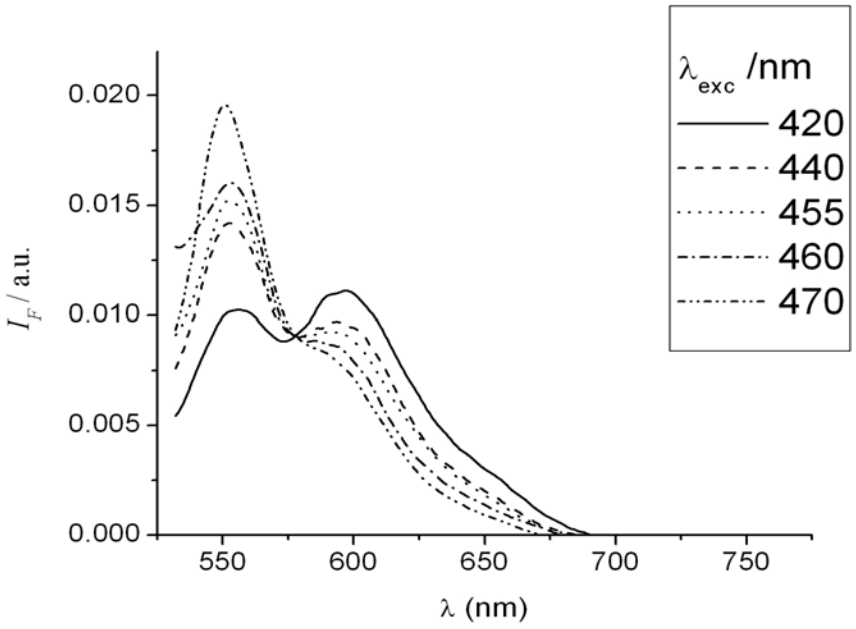


Fig. 4 Area normalized fluorescence emission spectra of alizarin in cyclohexane at different excitation wavelength.

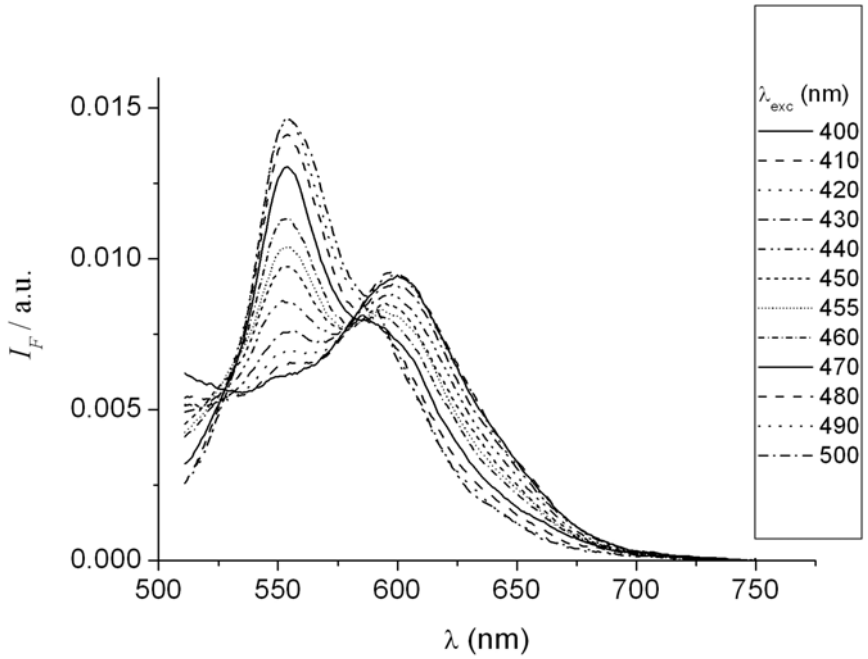


Fig. 5. Area normalized fluorescence emission spectra of Alizarin in dichloromethane at different excitation wavelength.

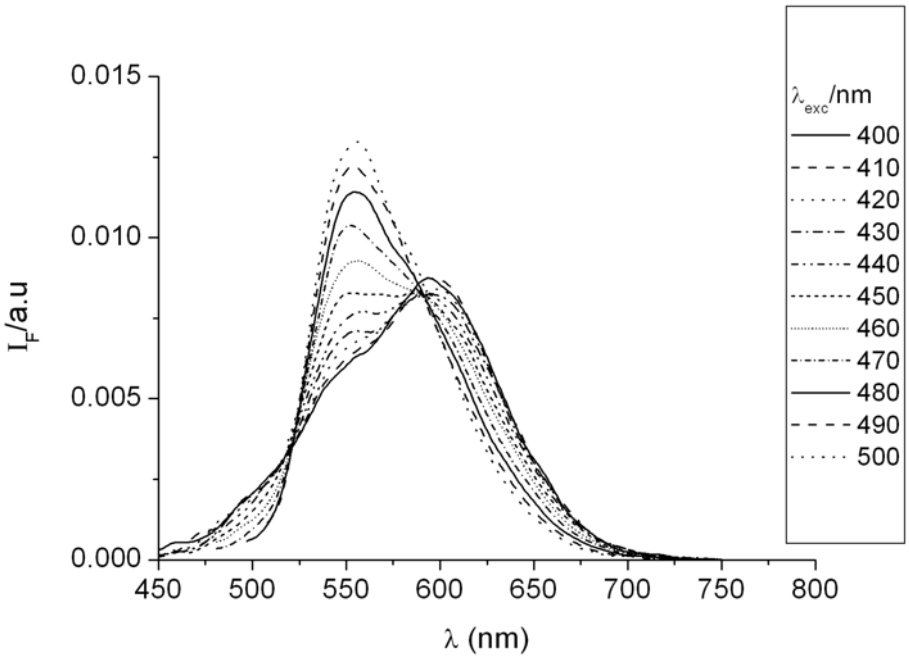


Fig. 6 Area normalized fluorescence emission spectra of Alizarin in 1,4-dioxane at different excitation wavelength.

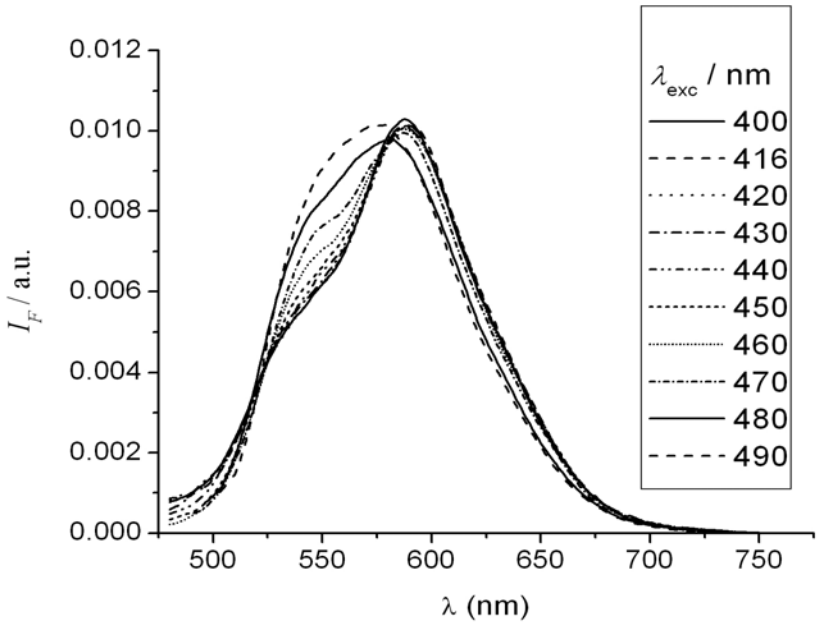


Fig. 7 Area normalized fluorescence emission spectra of Alizarin in ethanol at different excitation wavelength.

In ethanol the fluorescence emission spectra is different from what is observed in non-polar solvents and polar aprotic solvents. In ethanol, in contrast to aprotic solvents, the fluorescence intensity of the long wavelength band is greater than the fluorescence intensity of the short wavelength band at almost all fixed excitation wavelengths (Fig. 7) indicating that this solvent stabilizes the electronic structure responsible for this band. Even in methanol only the longer wavelength is observed [30]. The long wavelength fluorescence emission band in this solvent is blue shifted as compared to the position of this band in aprotic solvents. When excitation wavelength is fixed in the range (400-440) nm, the short wavelength emission band appears only as shoulder (Fig. 7). For excitation wavelength fixed in the range 460 – 490 nm, the short wavelength becomes intensified (Fig.7). Isoemission point in this solvent at about 588 nm also indicates the existence of two electronic structures in equilibrium. The variation of relative intensities of the two bands with excitation wavelength also indicates the dependence of emission spectra on excitation wavelength. The difference in the relative intensity of the two bands in aprotic and protic ethanol is the pronounced possibility of intermolecular hydrogen bonding in

ethanol that is not possible in non-hydrogen bonding solvents. In addition to the two-emission bands, a third emission band is also observed in this solvent at higher excitation wavelength which may be due to hydrogen bonding complexes or one of the tautomers of alizarin.

In both kinds of solvents except the difference in the relative intensities of the two bands, the band shapes and emission positions are almost the same indicating that the change in the electronic structure of this compound responsible for the observed bands in the excited state is insignificant.

5.1.3 Proton NMR-Spectra of Alizarin in Deutrated Chloroform and Deutrated DMSO

Another experimental method, proton NMR, is also used to identify the environment of the different types of proton in alizarin and structures present in the ground state [34]. From figure. 8, proton NMR spectrum of alizarin in deutrated chloroform, the protons of the two -OH groups are located at the two extreme peaks i.e. one at 6.3 and the other at 12.84. The up fielding of proton of –OH on the aromatic ring up to 6.3 is unusual, which may be attributed to the electron of oxygen atom is not delocalized with the ring system. Had the lone pair of oxygen of (O-13) delocalized with the ring system or proton of this group (H^{26}) (Fig.9) involved in the hydrogen bonding, this up fielding would have not been observed. On the other, the proton of the down fielded –OH (H^{19}) (Fig.9) group indicates the lone pair of oxygen is delocalized with the ring system. Hydrogen bonding between –OH closer to the carbonyl group and carbonyl oxygen is responsible for the down fielding this proton as shown in (Fig.11). The chemical shift position of the proton of –OH groups in DMSO is different from what is observed in deutrated chloroform (Fig.11). The –OH proton (H^{26}) is at about 11.8 ppm indicating that this proton is involved in hydrogen bonding or the lone pair of oxygen (O-13) is delocalized with the ring system.

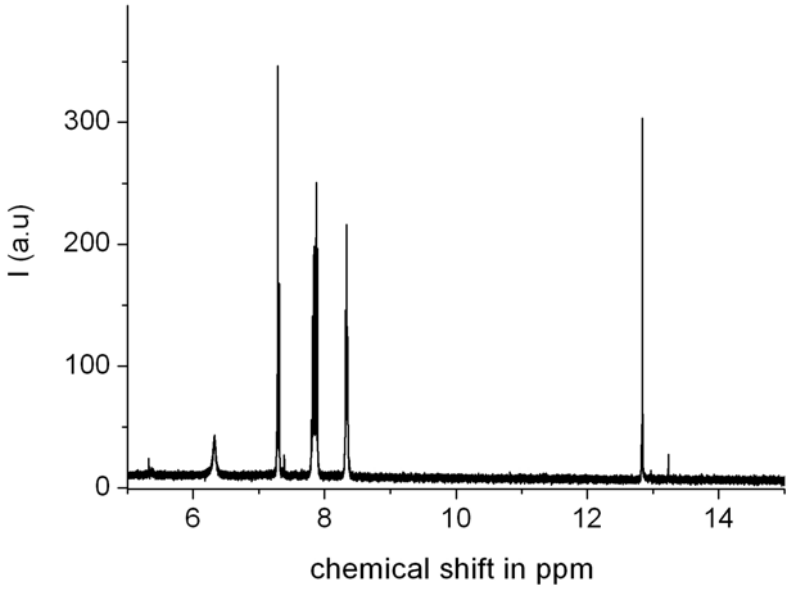


Fig. 8. Proton-NMR spectra of alizarin in $CDCl_3$.

Table. 1.Chemical shift of proton NMR in CCl_3D.

Protons	Chemical Shift (ppm)
H^{19}	12.867
$H^{20} = H^{21}$	7.874
$H^{23} = H^{22}$	8.350
H^{24}	7.290
H^{25}	7.833
H^{26}	6.330

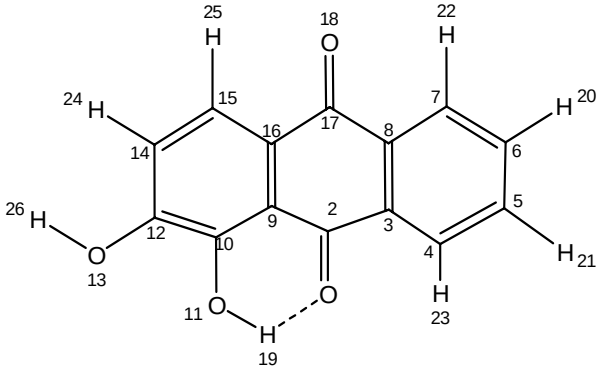


Fig. 9.Lebelled structure of alizarin.

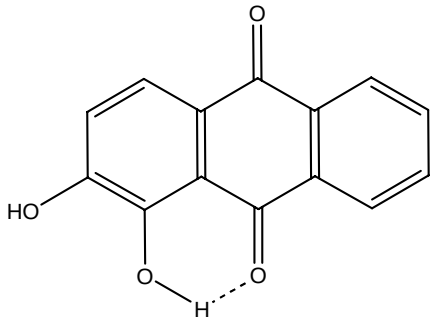


Fig. 10.Intramolecular Hydrogen-bonding in alizarin.

The chemical shifts of the other protons (proton on the benzene ring) are at the expected positions (6-9) in both of the solvents table.1&2. The difference in the position of one of the –OH protons (H^{26}) in the two solvents of different polarity may be attributed to the different interaction of the two solvents with alizarin. In DMSO, oxygen of DMSO may interact with proton of both –OH groups and hydrogen bonding interaction down field both protons. The down fielding of the –OH proton ($H^{19)}$ indicates this proton is highly mobile making the alizarin to exist in equilibrium with the other tautomer (Fig.11).

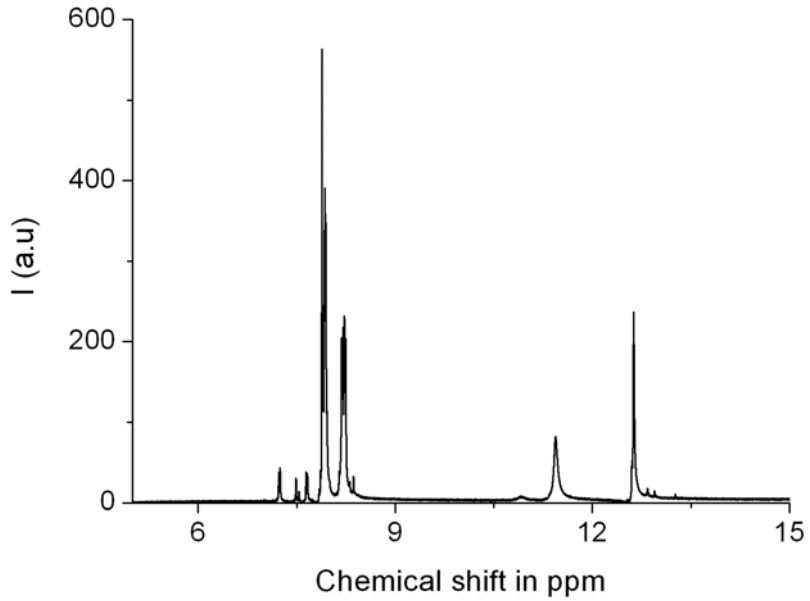


Fig. 11 Proton-NMR spectra of alizarin in deutrated DMSO.

Table.2. Chemical shift of proton NMR in deutrated DMSO.

Protons	Chemical shift in ppm
H^{19}	12.658
H^{20}	7.63
H^{21}	7.856
$H^{23} = H^{22}$	8.1
H^{24}	7.263
H^{25}	7.687
H^{26}	11.44

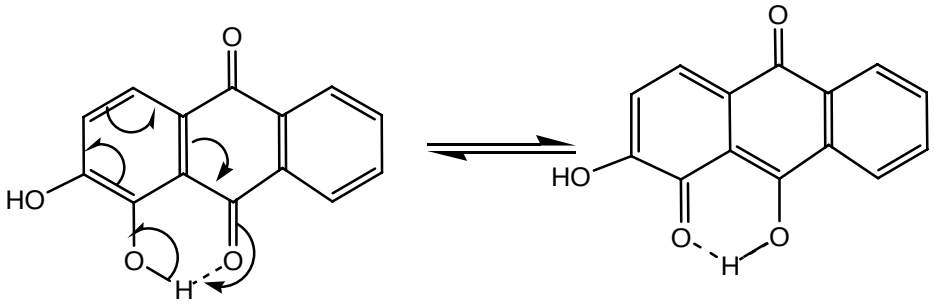


Fig.12.Tauatomerization of alizarin to give Al-3.

From experimental observations discussed above, some tautomerization and its mechanisms can be suggested. In aprotic solvents only intramolecular hydrogen bonding is expected to be one of the deriving forces for the species to be at equilibrium. Based on that and the experimental observations, the following structures are temporarily expected to be in equilibrium in aprotic solvents (Fig.13).

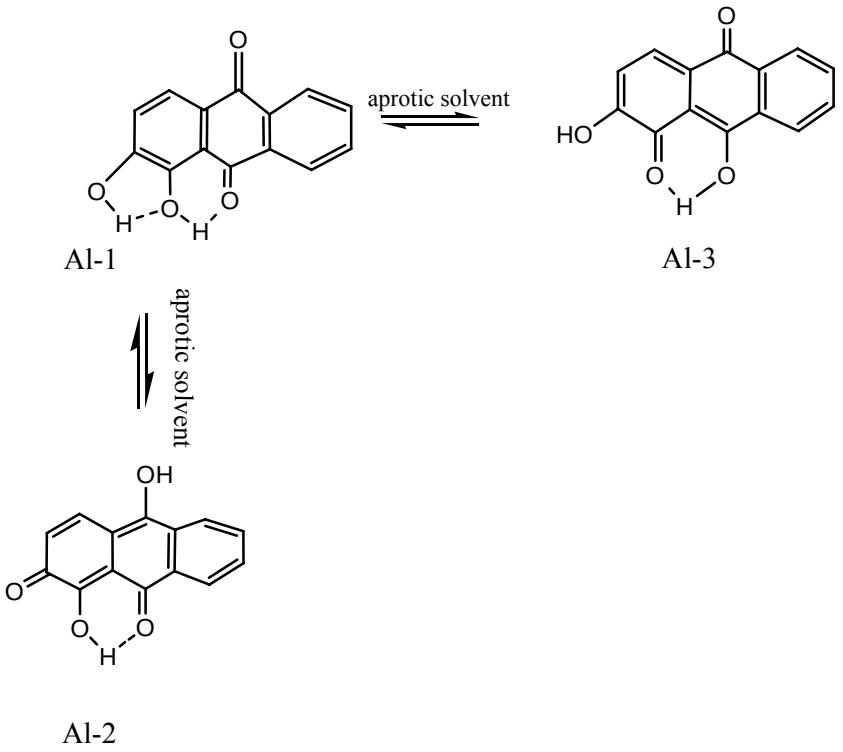


Fig.13. Alizarin and its tautomers expected to exist in equilibrium in aprotic solvents.

In fluorescence emission spectra it is only the presence of two species at equilibrium are clearly seen. So only one of the above equilibrium needs to be considered to be responsible for fluorescence emission spectra. The equilibrium leading to the formation of Al-2 indicates the proton of the –OH group responsible for its formation is mobile and the equilibrium leading to the formation of Al-3 indicates the proton of the –OH group leading to this formation is mobile. The proton NMR-spectra in deutrated chloroform (aprotic solvent) supports the second alternative.

Based on fluorescence emission spectra and inter-or intra-molecular hydrogen bonding in ethanol the following structures are temporarily expected to be in equilibrium.

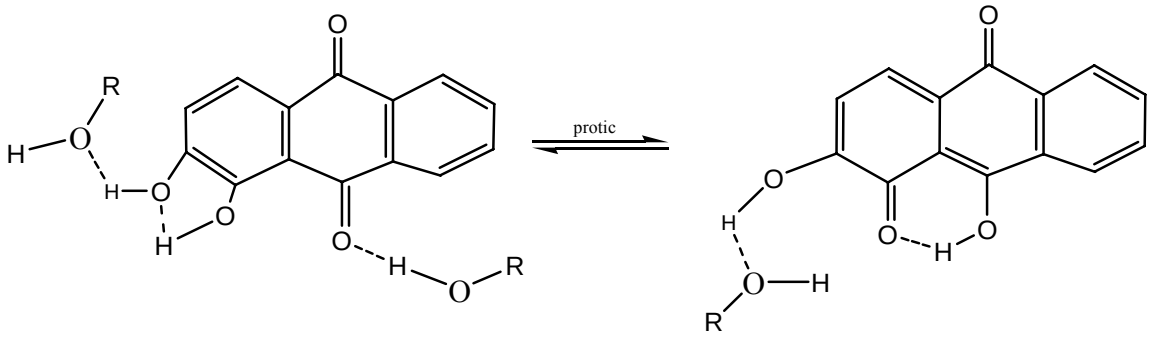


Fig.14. Alizarin and its tautomers that may exist in equilibrium in ethanol (R =CH_3CH_2-).

The proton-NMR spectrum of alizarin in DMSO indicates the involvement of both –OH groups in hydrogen bonding down fielding both protons to (11.44 &12.658) (table.2). Attributed to the involvement of the carbonyl oxygen (O) in intermolecular hydrogen bonding (Fig.14), the tautomerization reaction leading to the product in ethanol is slow indicating that the reactant is more stable than the product. Thus, according to this concept Al-1 is more predominant than Al-3 in ethanol. If this is true, Al-3 is more predominant in aprotic solvent since the relative intensities of the two florescence bands corresponding to the concentration of different electronic structures are the reverse of each other in the two types of solvents (aprotic and protic). In 1,4-dioxane though intermolecular hydrogen bonding is possible it doesn't interact with oxygen of carbonyl group, as it doesn't contain mobile protons. Consequently, in this solvent what is observed in non-hydrogen bonding solvent is expected.

5.1.4 Influence of $_P$H on fluorescence spectra of Alizarin

Fluorescence emission, excitation and absorption spectra of many compounds were found to be sensitive to the pH of the media as described elsewhere in the background part [2,14,36,37]. In order to see the effect of pH on fluorescence emission spectra, KOH and H_2SO_4 were added to alizarin solution of cyclohexane and ethanol. It was reported that only addition of base was found to change the absorption spectrum of alizarin i.e. in acidic media absorption spectrum of alizarin is not change. Since different properties

were observed in aprotic and protic solvents, this heading is treated under two sub-headings.

5.1.4.1 Influence of pH on fluorescence emission of spectra of Alizarin in cyclohexane

Addition of H_2SO_4 to alizarin in cyclohexane does not change the position of fluorescence emission bands rather it enhanced the intensity of the bands by increasing the solubility of alizarin in cyclohexane (Fig.15). On the other hand, addition of few drops of KOH to alizarin in cyclohexane changed the emission spectrum in such a way that only the long wavelength emission band is observed, which is blue shifted by about 10 nm as compared to the position of this band in cyclohexane (Fig.15) that is in the region of the long wavelength emission band of alizarin in ethanol. The variation of fluorescence emission spectra with excitation wavelength in acidic media is almost the same with what is observed in cyclohexane (Fig.16). Thus, acidification does not affect the electronic structure of the emitting and absorbing states in cyclohexane. The influence of bases on emission spectrum is also expected as bases change the nature of absorbing species by deprotonation of the -OH protons. In cyclohexane, addition of KOH caused the long wavelength emission band to be more intensified. This may be due to stabilization of the electronic structure responsible for this band by the –OH groups. No new band is observed in this media, only the electronic structures responsible for the long wavelength emission band is identified in the fluorescence emission spectrum at excitation wavelength of 470 nm. This observation indicates in this media at this excitation wavelength only the electronic structure responsible for the long wavelength emission band is present in excess in equilibrium with the anionic species produced by deprotonation of the –OH protons. The emission bands corresponding to the anions are not observed at excitation wavelength of 470 nm. This implies two things; one at this excitation wavelength no anion is excited and emissions are not expected or the anion that is excited at this excitation wavelength may not fluoresce.

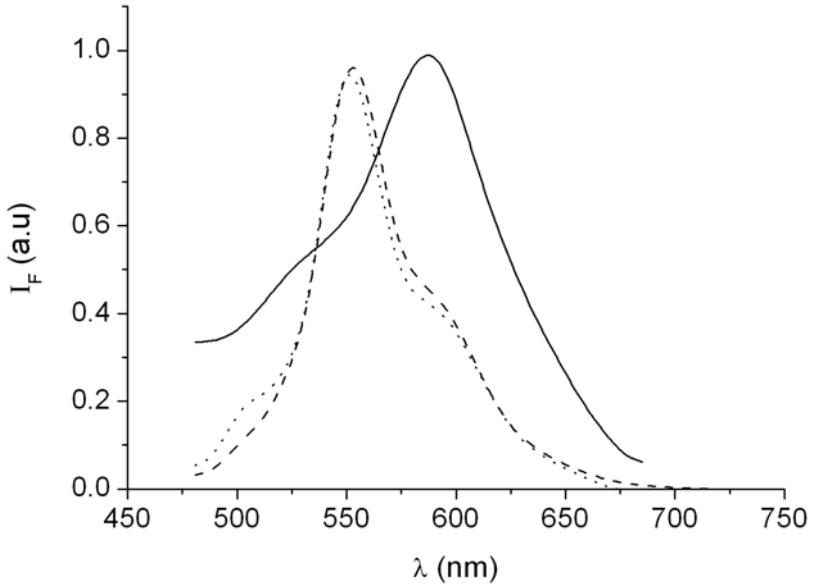


Fig.15. Influence of pH on fluorescence spectra of alizarin in cyclohexane at excitation wavelengths of 470 nm in basic cyclohexane (—), acidified cyclohexane (‾‾) and free cyclohexane (···).

In basic media in cyclohexane the neutral species may exist in equilibrium with anionic species at lower KOH concentration. But at higher KOH concentration only the anionic species are expected (not found in my experimental part). Since with increasing electronic density absorption shifts to the red and absorption and emission are mirror image of each other, the bands (excitation and emission) corresponding to the anions are expected to be at higher wavelength region. In the mechanism (a) (Fig.17) deprotonation is relatively difficult as both protons are involved in hydrogen bonding. Mechanism (b) is the probable deprotonation in basic cyclohexane media. If (b) is the possible deprotonation mechanism in this media, the band corresponding to the tautomer Al-3 does not exist even at lower –OH concentration. Only the bands corresponding to the anion from Al-3 and the one corresponding to alizarin (Al-1) is expected in both excitation and emission spectra.

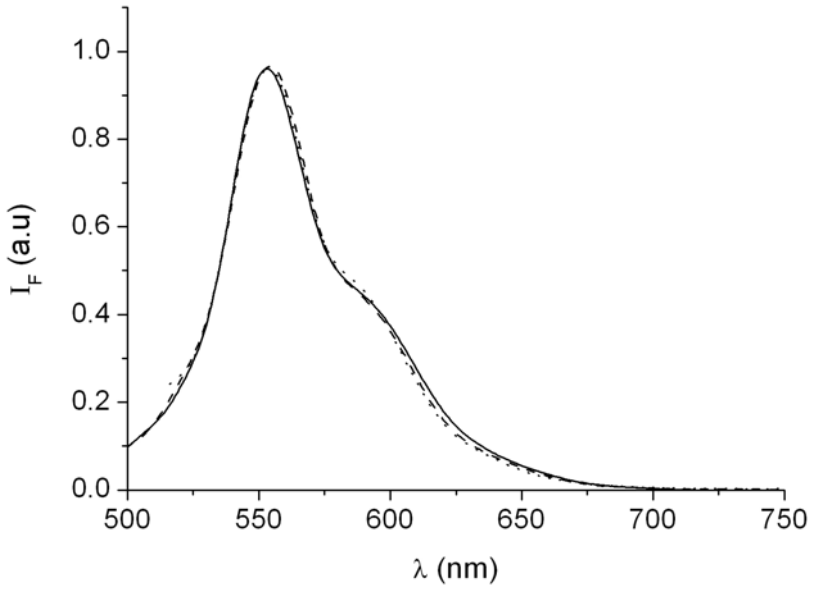


Fig.16. Influence of excitation wavelength on fluorescence emission spectra of alizarin in acidified cyclohexane at λ_{ex} = 470 nm (—), λ_{ex} = 480 nm (---) and λ_{ex} = 500 nm (···) in acidified cyclohexane.

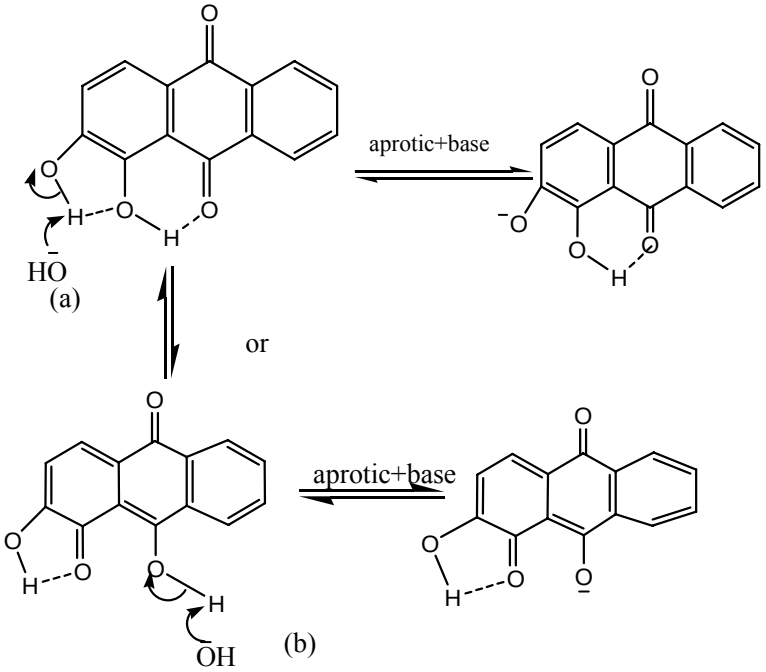


Fig.17. Compounds that may exist in equilibrium in basic cyclohexane.

5.1.4.2 Influence of pH on Fluorescence Spectra of Alizarin in Ethanol

In cyclohexane it was only addition of bases that altered the fluorescence emission spectrum of alizarin. The same trend is observed with acid in ethanol. Almost no change is observed up on addition of acid to ethanol (Fig.18). A different property is observed if KOH is added to ethanol solution of alizarin (Fig.18). In the basic media the short wavelength emission band is blue shifted to about 522 nm, which may be attributed to the formation of new species or the effect of the media on the original emitting species. In addition to this band, a weak red shifted fluorescence band is observed around 650 nm and (Fig.18). Thus, different chemical species are expected in the two solvents i.e. hydrogen bonding solvent (ethanol) and non-hydrogen bonding solvents (cyclohexane) if base is added to them.

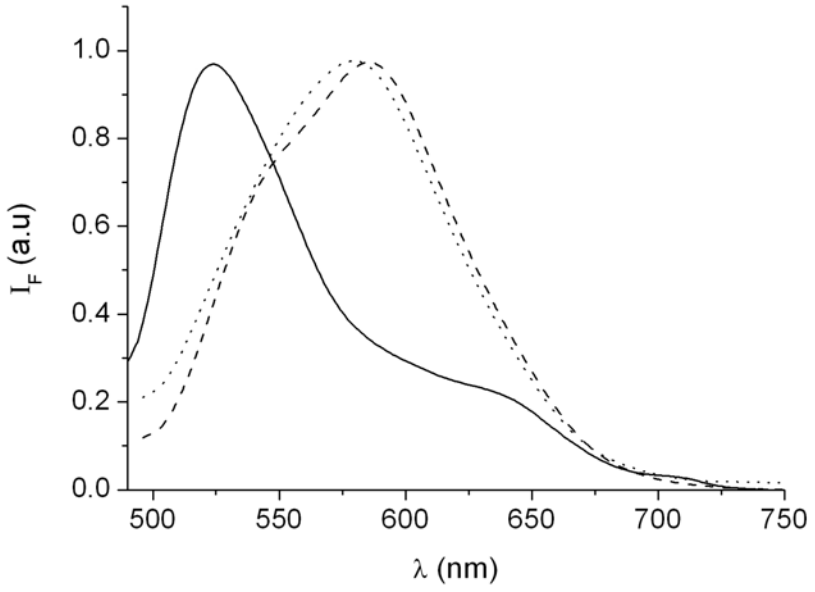


Fig.18 Influence of pH on the fluorescence spectra of alizarin in ethanol at λ_{ex} = 470 nm in basic ethanol (KOH) (—), free ethanol (‾‾) and acidified ethanol (H_2SO_4) (···).

The longer wavelength emission band may be the one corresponding to the anionic substance produced in this media or forbidden transitions. The short wavelength

emission band in this media is not expected to correspond to the anions. With increase in electron density for a compound, absorption bands show red shifting. Since absorption and emission are mirror image of each other, emission bands also red shift with increase in electron density (in our case deprotonation increases electron density of alizarin). The following are the proposed mechanisms for the deprotonation process in basic ethanol media.

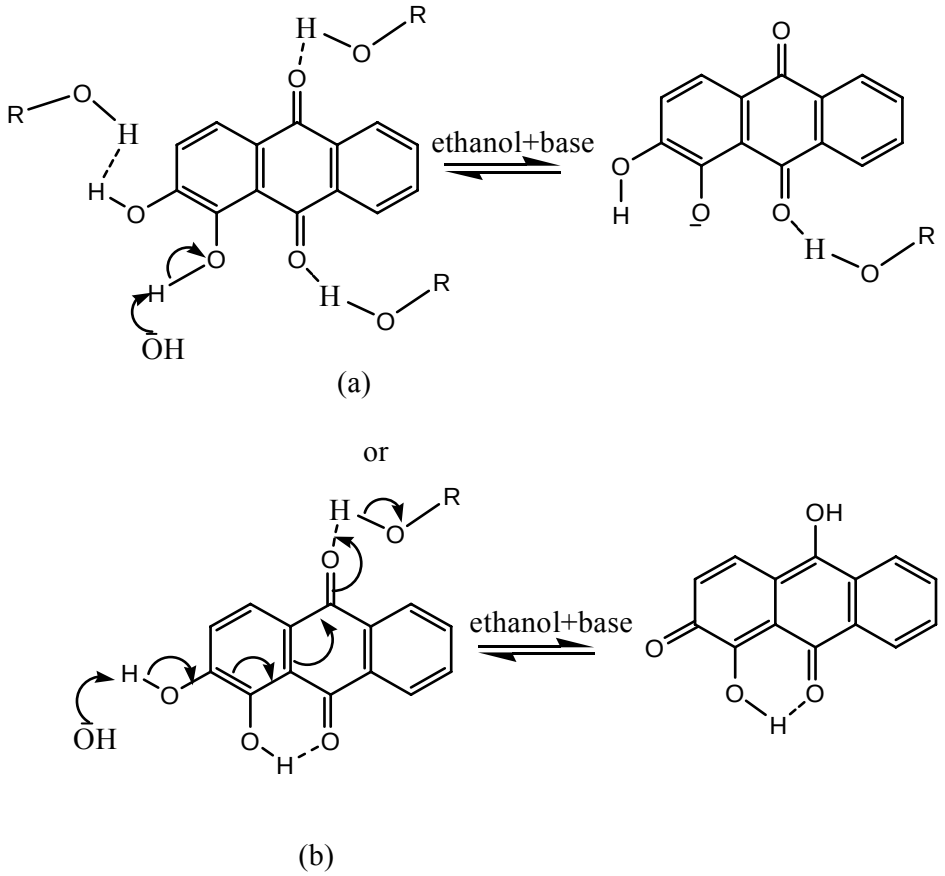


Fig.19. Compounds that may exist in equilibrium in basic ethanol.

The mechanism in figure.20 shows not only the deprotonation leading to anion; it also shows base catalyzed reaction leading to the formation of one of the alizarin tautomers. The blue shifted fluorescence band may correspond to this generated tautomer (Al-2). And the long wavelength fluorescence band in this media may be the one expected for the anion.

Different fluorescence bands are observed up on addition of base to the solution of cyclohexane and ethanol. This difference may be due to the different possible site of deprotonation in the two solvents as shown in (Fig. 17 &19).

5.1.5 Influence of concentration on Fluorescence Spectra of Alizarin in 1,4-dioxane and ethanol.

As shown in the figure.20, fluorescence signal is directly proportional to concentration of the absorbing species. This direct proportionality is expected only up to certain ranges of concentration. In solvents in which alizarin shows significant solubility, concentration may affect the fluorescence intensity, position or the shape of the bands. Though in the figure.20 a direct proportionality is observed between concentration and fluorescence signal, it is difficult to say at all concentration fluorescence signal and concentration are directly proportional for this compound. In solvent in which alizarin is sparingly soluble concentration effect need not be expected. Normalized spectrum of alizarin in 1,4-dioxane at different concentrations shows band form is not changed with concentration in the rang of concentrations used (Fig.21).

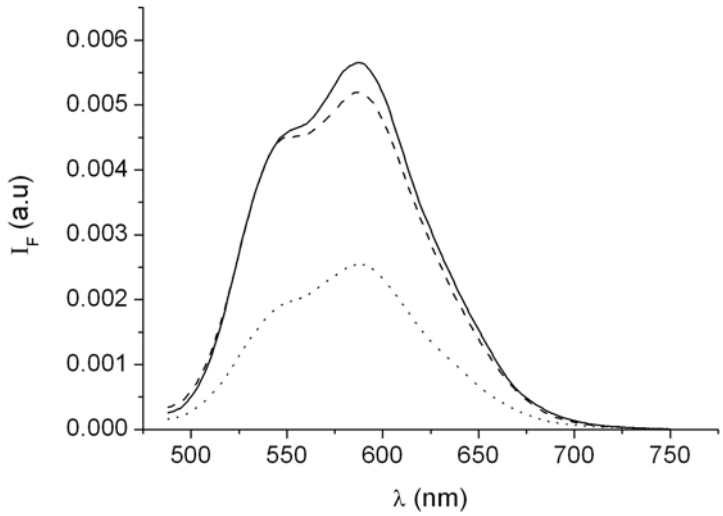


Fig.20 Influence of concentration on fluorescence emission spectra of alizarin in ethanol at excitation wavelength of 470 nm, at 1.5 $x10^{-5}$ (—), $7.5x10^{-6}$(---) and $3.75x10^{-6}$ (···).

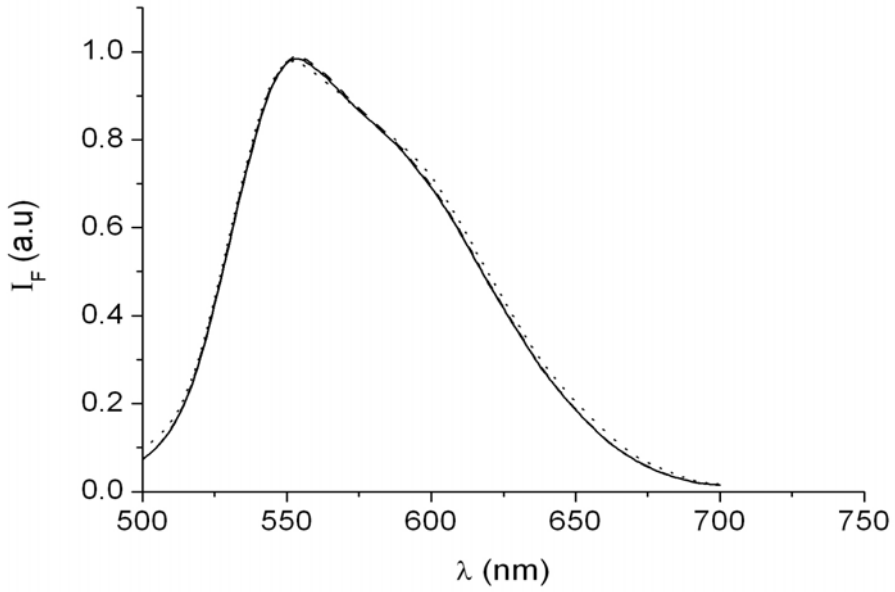


Fig.21. Influence of concentration on the fluorescence emission spectra of alizarin in 1,4-dioxane at excitation wavelength of 470 nm, 1.5 $x10^{-5}$ (—), $7.5x10^{-6}$(---) $3.75x10^{-6}$ (···).

In polar (both protic and aprotic) solvents there is an increase in the intensity of absorption, emission and fluorescence emission band when concentration of alizarin is held between certain ranges. Above a critical value of concentration, especially fluorescence band is affected due to different processes taking place with varying concentration. At higher concentration excimer (excited state dimmer) or exciplex formation may alter the bands. Up to the concentration we considered no effect of it on fluorescence emission band, shape and intensity of the bands were observed (Fig.20&21).

5.2 Calculation of certain Properties of Alizarin and its Tautomers by Computational Methods

In addition to experimental methods described above theoretical methods were used to study the fluorescence and tautomerization character of this compound. Computational methods were used to calculate some properties of molecules in both ground and excited state as described in the background part. In our case three computational methods were

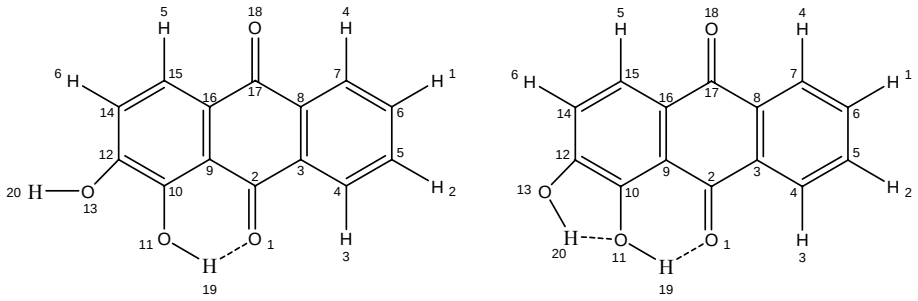
employed to calculate properties of the molecules in ground state. ZINDO and TDSCF were used to predict the properties in the excited state. HF optimizes the structure of the molecules by using basis set STO-3G then the frequency and NMR of the four tautomers were calculated by this method using the same basis set. B3LYP of DFT is also used to optimize the structure of alizarin and its tautomers and to calculate the above molecular parameters. Table.3 shows total internal energy decreases in the order (Al-1) to (Al-4) and dipole moment of the optimized structure of alizarin and its tautomers is in the order (Al-1)<(Al-3) <(Al-2) <(Al-4) calculated by the two computational methods Hartree-Fock (HF) by using basis set STO-3G and B3LYP of density functional theory (DFT) by using basis set STO-3G. In this table, Gibbs free energy of the optimized structures is also calculated with HF/STO-3G. These results may enable one to predict the relative polarity and stability of alizarin and its tautomers. Theoretically, more polar structure is expected to exist in more polar solvents since more polar tautomer is stabilized in more polar solvents due to the various types of stabilizing interaction i.e. electrostatic, hydrogen bonding, dipole-dipole interactions and etc.

Table.3. Energy and dipole moment of optimized structure calculated by HF/STO-3G and DFT/STO-3G in the ground state.

Tautomer	E(HF) (H/particle)	E(DFT) (H/particle)	μ DFT (Debye)	μ (HF) (Debye)	G(HF) (H/particle)
Al-1 (a)	-823.6734	-828.4162	0.657	1.955	-823.4955
Al-1 (b)	-823.6802	-828.4318	2.263	2.668	-823.5020
Al-2	-823.6331	-828.4094	5.169	5.317	-823.4558
Al-3	-823.6266	-828.3854	4.734	4.193	-823.4503
Al-4	-823.6213	-828.3671	6.553	6.417	-823.4588

The two methods used in calculating dipole moment, total energy gave similar relative results for the tautomers as shown in table.3. The difference is that DFT optimized the alizarin and its tautomers more as it gave less total energy for all of the tautomers. In table.3 the relative stabilities (Gibb`s free energy) of each tautomer in the gas phase is described; accordingly alizarin Al-1 (b) is the most stable structure. From this table an

interesting point to be recognized is the differences between the stability of the two conformational isomers Al-1 (a) and Al-1 (b) (table.1) i.e. Al-1 (b) is more stable than Al-1 (a) (table.3). Gibbs free energy of the tautomers enable one to predict which tautomerization reaction is more feasible for the formation of the tautomers from alizarin.



Al-1 (a) Al-1 (b)

Fig. 22.Intramolecular hydrogen bonding in alizarin, Al-1 (a) and Al-1 (b).

Since alizarin is polar compound, its interaction with no-polar solvent is less and its properties in these solvents and in the gas phase are expected to be almost similar.

Table.4. Energy and dipole moment of optimized structure of alizarin and its tautomers calculated by DFT /B3LYP level using the basis set 6-31G* in the ground state.

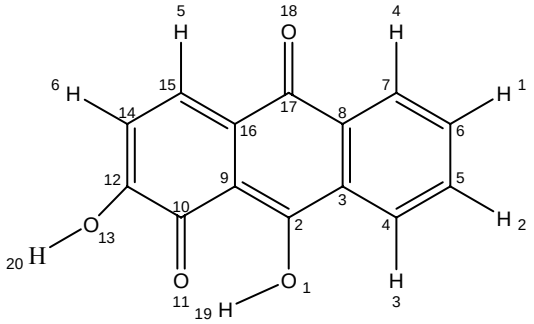
Tautomer	E (H/particle)	μ (Debye)
Al-1 (a)	-839.22142515	0.9973
Al-1(b)	-839.4878649	2.0339
Al-2	-839.19629261	8.4528
Al-3	-839.19011820	5.1461
Al-4	-839.16896770	9.6691

Higher order basis set of DFT is also used to optimize the structure of alizarin. As indicated in table.4, higher basis set (6-31G*) gave more optimized structure indicated by lower total energy for all of the tautomers. A Proton-NMR was also calculated for alizarin and its tautomers by using 6-311+G* basis set (table.5). According to the results in this table one of the proton of the –OH groups is down fielded (H^{19}) to about 13.36

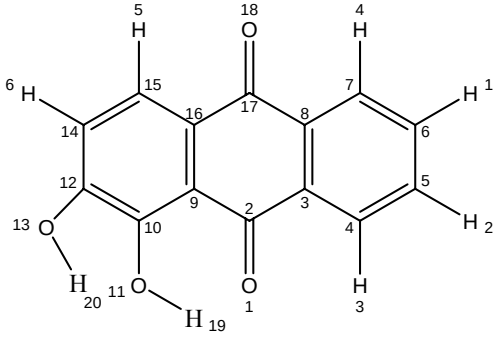
ppm for Al-1, 15.5 ppm for Al-2 and 17.47 ppm for Al-3 which is almost similar to the results obtained experimentally in both CCl_3D and DMSO. Chemical shift for the other proton (H^{20}) is relatively up fielded to about 4.36 ppm for Al-1, 6.22 ppm for Al-2 and 8.4 ppm for Al-3. The relatively up fielding of this proton was also observed experimentally in CCl_3D. These results also indicate intramolecular hydrogen bonding is responsible for the down fielding of one the –OH protons (H^{19}). The coinciding of computational and experimental result described above is an evidence for the high precision of computational technique in calculating molecular parameters i.e. bond length, bond angles, total energy, Gibbs free energy, dipole moments, etc. Since the chemical shift position of Al-4 calculated by this method is far from the experimental results, the existence of this compound is not expected with the other tautomers. The difference between ^1H NMR of alizarin calculated by DFT/B3LYP/6-311G* in deutrated DMSO and gas phase is similar to the difference between 1H NMR of alizarin in deutrated DMSO and deutrated chloroform recorded experimentally except the chemical shift position of H^{26}. The position of this proton in deutrated DMSO is at about 11.4ppm experimentally and 6 ppm calculated by the method mentioned.

Table.5. Chemical shift in ppm of proton of alizarin and its tautomers obtained by (DFT/6-31G*) and their proton-NMR calculated by DFT /B3LYPlevel using the basis set (6-311+G *) in the ground state in the gas phase.

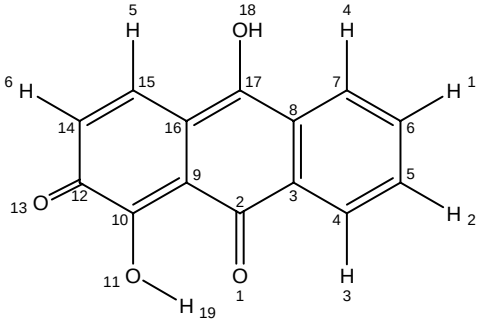
	H^1	H^2	H3	H^4	H^5	H^6	-OHproton	-OHproton
Al-1	8.0	7.9	8.8	8.7	8.2	6.9	13.3 H^{19}	4.3 H^{26}
Al-2	7.9	7.8	9.0	7.6	8.2	6.6	15.5 H^{19}	5.0 H^{18}
Al-3	8.7	8.6	8.6	8.8	8.8	7.8	17.5 H^{19}	8.4 H^{20}
Al-4	7.9	7.9	8.1	8.0	8.5	6.6	5.9	4.5



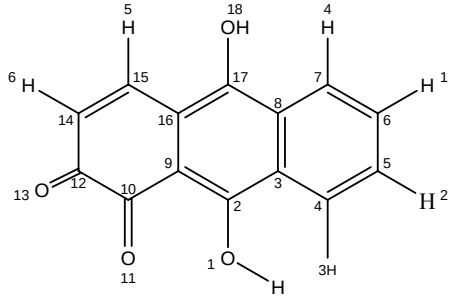
Al-3



Al-1 (b)



Al-2



Al-4

Fig. 23.Lebelled structure of alizarin (Al-1), Al-2, Al-3 and Al-4.

Table.6. Chemical shift in ppm of proton of alizarin and obtained by (DFT/6-31G*) and their proton-NMR calculated by DFT /B3LYPlevel using the basis set (6-311+G *) in the ground state in Deutrated DMSO.

	H^1	H^2	H^3	H^4	H^5	H^6	-OHproton	-OHproton
Al-1	7.6	7.6	8.0	7.9	7.4	6.8	11.7 H^{26}	6.0 H^{25}

Table.7. Hydrogen Bond lengths (--) and normal covalent bond length (-) of optimized structure of Al-1 (a) and Al-1 (b) by B3LYP of DFT using the basis set 6-31G*.

Bond	Bondlength (Å) Al-1 (a)	Bondlength (Å) Al-1 (b)
H (19)-- O (1)	1.68283	1.68802
C (10)-O (11)	1.33602	1.34904
H (19)-O (11)	0.99526	0.99774
C (2)-O (1)	1.24578	1.24647
H (20)-O (13)	0.97045	0.97521
C (12)-O (13)	1.35686	1.35283
H (20)--O (11)	3.59902	2.10107
H (19)--O (13)	3.61838	

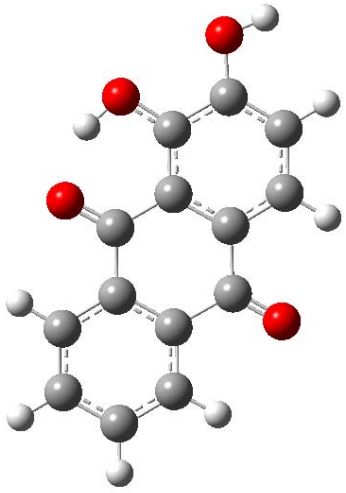
In order to distinguish the relative strength of the six membered and five membered hydrogen bonding (Fig.22), bond length calculation was performed by DFT/6-31G*(table.7). Hydrogen bonding occurs only when hydrogen bonding acceptor and donor are in certain range of interatomic distances the maximum being (3.5 Å). According to (Baker and Hubbard, 1984) the maximum interatomic distance between hydrogen and acceptor for hydrogen bond to exist is 2.5 Å. The result shows for Al-1 (b) the hydrogen-bonding length (H19---O1) is about 1.688 Å and the hydrogen bonding length (H20---O11) is about 2.1011 Å indicating the first hydrogen bonding is stronger. The greater strength of the first hydrogen bonding may be also attributed to the greater stability of the six-ring system as compared to the five-ring system. Thus, this result coincides with the stability expectation. The bond length of H19-O11 (0.99526 Å) is greater than the bond length of H20-O13 (0.97045 Å) since the stronger (H19---O1) hydrogen bonding weakens H20-O13 bond more than (H20---O11) hydrogen bonding

weakens H20-O13 bond. Considering the bond lengths C10-O11 (1.33602) and C 12-O13 (1.35686 Å), the relatively greater strength of C10-O11 may be due to more delocalization of the lone pair of electrons on the oxygen of this bond with the ring system as compared to the delocalization of the lone pair of electrons on the oxygen of C 12-O13 with the ring system. ZINDO and TDSCF were used to calculate the excitation energy of alizarin and its tautomers (table.8).

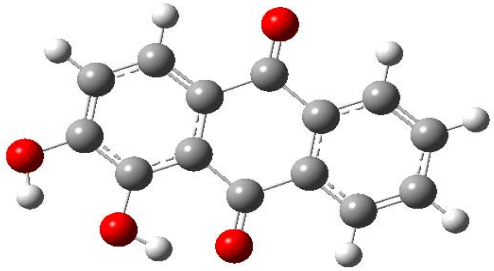
Table.8. λ_{max} and dipole moment of alizarin and its tautomers in the first singlet excited state calculated by ZINDO and TDSCF.

Compound	λ_{max} (nm) ZINDO	λ_{max} (nm) TDSCF	Dipole moment (Debye)
Al-1	472.5	433.5	78.5
Al-2	535.0	510.3	86.2
Al-3	484.0	533.5	83.3
Al-4	591.0	589.3	94.2

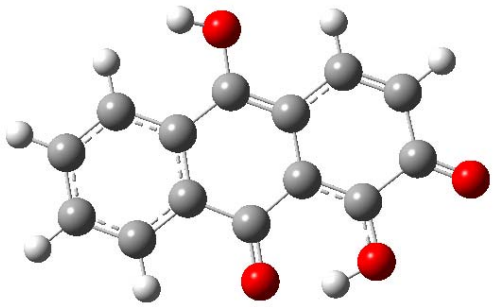
Excitation wavelength of alizarin and (Al-3) calculated ZINDO is almost equal to the excitation wavelength observed in the emission spectrum of alizarin in almost all of the solvents used in the experimental part (Fig.4, 5,6&7). Excitation at 470 nm is responsible for the fluorescence emission band at 595-598 in aprotic solvents and at 588-590 nm in protic solvent described in the experimental part (Fig.4, 5, 6&7) the difference being attributed to hydrogen bond interaction in protic solvents. On the other hand excitation at about 486 nm (Al-3) gives fluorescence emission band at 550 nm as discussed else where above and (Fig. 4). The results in table.7 also describe the relative polarities of alizarin and its tautomers in the excited state by calculating their dipole moments.



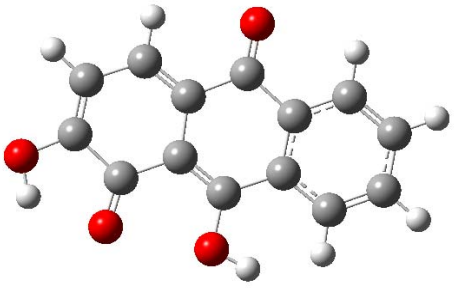
1,2-dihydroxyanthracene-9, 10-dione (Al-1)-a



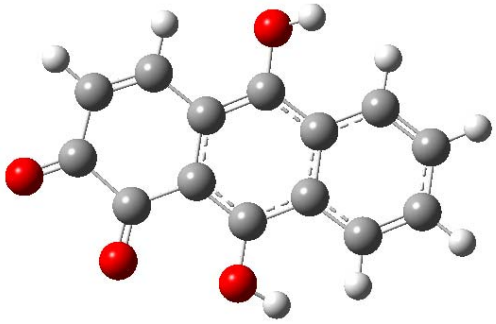
1,2-dihydroxyanthracene-9, 10-dione (Al-1)-b



1,10-dihydroxyanthracene-2, 9-dione (Al-2)



2,9-dihydroxyanthracene-1, 10-dione (Al-3)



9,10-dihydroxyanthracene-1, 2-dioxane (Al-4)

Fig.24. Optimized structure of alizarin and its tautomers by DFT using the basis set 6-31G*.

6. Conclusion

Both experimental and theoretical methods were used to study the spectral and tautomerization properties of alizarin. Experimentally, the effect of solvent of different polarity, the effect of concentration and $_P$H of the media on the fluorescence emission spectra and tautomerization processes were studied. Polarities of solvents were found to change the relative intensities of the two fluorescence bands i.e. the position of these bands don't significantly change with solvent polarity. In acidic media no significant changes on both absorption and fluorescence emission spectra of the compound were observed. In basic media, both absorption and fluorescence emission were changed attributed to the deprotonation of the –OH protons.

Excitation wavelength dependence of emission spectrum indicated the presence in the ground state of two different chemical species excited at different wavelength and emitting at their corresponding wavelengths. Three of the tautomers were proposed to

select two of them which are responsible for the observed fluorescence emission bands observed the experimentally.

Different mechanisms for the equilibrium between the two species were proposed based on the spectral shifts in solvents of different polarity, molecular structure of alizarin and pH effects. The effect of solvent polarity on proton-NMR spectrum also gave the information for the existence of two species in equilibrium. Since this compound can exist in more than one tautomerization equilibria, confirming a single equilibrium is difficult. But what is experimentally observed indicated the greater probabilities of one the equilibria that does not imply the other equilibria are not possible. So based on the observations above the equilibrium between alizarin and one of its tautomers (Al-3) better explains what is observed experimentally in different media. Computational methods used enabled us to identify the relative probabilities of the different types of equilibria. Proton-NMR of the alizarin and its tautomers calculated by DFT/B3LYP with basis set 6-311+G* revealed the contribution of the intramolecular hydrogen bonding to the equilibrium between species. And computationally the relative polarities and stabilities of alizarin and its tautomers in the gas phase were revealed. An important result obtained by this method is excitation energy of alizarin and its tautomers calculated by ZINDO and TDSCF. Excitation energy of alizarin and Al-3 are closer to two of the bands observed in the excitation spectrum indicating that these two species are in equilibrium. So what is proposed from experimental observation synchronized with theoretical calculation.

7. Reference:

1. A. Polimeno, A. Barbon, and P. L. Nordio, J. Phys. Chem., 98, 12158-12168, ***1994.***
2. M. Balon, C. Carmona, M. A. Munoz, Chem. Phys., 302, ***2004.***
3. C. Yao, H. B. Kraatz , R. Steer, Photochem. Photobiol. Sci., 4, 191-199, ***2005.***
4. P. Purkayastha , N. Chattopadhyay, Phys. Chem. Chem. Phys., 2, 203-210, ***2000.***
5. A. M. G. Campana, F. A. Barrero and M. R. Ceba, Analyst. 117, ***1992***.
6. M. Kagayama, Y. Sasano, H. Satos, S. Kamakura, K. Motegi and I. M izoguchi, J. Anathomy and Embryology. 233-238, ***2004***.
7. A. Maliakal, G. Lem, N. J. Turro, R. Ravichandran, J. C. Suhadolnik, A. D. Debellis, Mervin G. Wood , and J. Lau, J. Phys. Chem. A., 106, 7680-7689, ***2002.***
8. I. Szydlowska, A. Kyrychenko, A. Gorski, J. Waluk, J. Herbich, Photochem. Photobiol. Sci., 2, 187-194, ***2003.***
9. J. Dobkowski, W. Retting and J. Waluk, Phys. Chem. Chem. Phys., 4, 4334-4339, ***2002.***
10. S. Zilberg, Y. Haas, J. Phys. Chem. A., 106, 1, ***2002***.
11. R.Ghanem, C. Carmona, P. Guardado, M. Munoz, M. Balon, J. Photochem. Photobiol. A, 129, 57-64, ***1999***.
12. M. Halder. P. K. Chowdhury, M. S. Gordon, J. W. Petrich, K. Das, J. Park, Y. Alexeev, A., photoChem., 28, ***2005***.
13. M. Panda and J. Chandrasekhar. J. Phys. Chem. A., 103, 6257-6262, ***1999.***
14. M. Balon, M. A. Munoz, C. Carmona, Chem. Phys., 299, 67-77, ***2003***.
15. J. D. Walson, Prac. Nat. Acad. Sci., USA, 69, 9, 2488-2491, ***1972.***
16. S. Maheshwari, A. Chowdhury, and N. sathyamurthy, M. Panda and J. Chamdrasekhar, J. Phys. Chem. A., 103, 6257-6262, ***1999***.
17. A. S. klymchenko and A P. demchenko, Phys.Chem. Chem. Phys., 5, 461-468, ***2003***.
18. Z. R. Grabowski, pure and Appl. Chem., 65(8), 1751-1756, ***1993.***
19. T. L. Arbeloa, F. L. Arbeloa, M. J. Tapia, I. L. Arbeloa, J. Phys. Chem. 97, 4704-4707, ***1993.***

20. F. Pine, M. J. Melo, H. Santos, J. C. Lima, I. Abreu, R. Ballardini and M. Maestri, New J. Chem., 1093-1098, ***1998***.
21. C. Reichardit, Solvent and solvent effect in organic chemistry, 3rd edition, WILLEY-VCH, Weinheim, 329-387, ***2003***.
22. D. A. McQuarrie, Quantum Chemistry, Vinod Vasishtha, New Delhi, 437-479, ***2003***.
23. B. Valeur, Molecular Fluorescence, Willey-VCH, Weinhein, ***2000.***
24. M. G. kuzmin, Pure and Appl. Chem., 65(8), 1653-1658, ***1993***.
25. J. R.Lakowicz, principles of Fluorescence spectroscopy, 2nd edition, Kluwer Acadamic Plenum press, New York, ***1999.***
26. J. Konijnenberg, A. H. Huizer and C. A. G. O. Varma, J. Chem. Soc., Faraday Trans., 85(2), 1539-1551, ***1989***.
27. S. Kapelle, W. Retting and R. Lapouyade, Photochem. Photobiol. Sci., 1, 492-499, ***2002***.
28. N. S. Domingues, Jr. C. Krug, P. R. Livotto and V. Stefan, J. Chem. Soc., Perkin Trans. 2, ***1997***.
29. S. Mente and M. Maroncelli, J. Phys. Chem. A, 102, 3860-3876, ***1998***.
30. R. Huber, S. Sporlei, J. E. Moser, M. Gratzel, and J. wachtveitl, J. Phys. Chem. B, 104, 8995-9003, ***2000***.
31. S. Schneider, O. Schmitt, G. Brehm, M. Reiher, P. Matousek and M. Towrie, Photochem. Phtotobiol. Sci., 2, 1107-1117, ***2003***.
32. F. Ying WU, L. MA, and Y. Boa JIANG, Japan Soc., Ana. Chem., 17, ***2004***.
33. G. R. Phillips and S. Georghiou, Biophys. J., 65(2), 918–926, ***1993.***
34. C. J. Jalink, W. M. Vaningen, A. H. Huizer and C.G. O. Varm, J. Chem. Soc. Faraday, 87, 1103-1108, ***1991***.
35. A. L. Weis, Z. Porat and Z. Luz, J. Am. Chem. Soc. 106, 8021-8024, ***1984***.
36. C. L. Dunford, G. J. Smith, E. E. Swinny and K.R. Markham, Photochem. Photobiol., Sci., 2, 611-615, ***2003***.
37. A. P. Monkman, M. Halim, I. D.W. Samuel, and L. E. Horshburgh, J. Chem. Phys., 109, 10372-10378, ***1998***.