Photophysical aspects of biological photosensitizer Kynurenic acid from the perspective of experimental and quantum chemical study

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HIGHLIGHTS

- Kynurenic acid (KA), an endogeneous and neuroactive NMDA receptor antagonist.
- KA is an end product of "kynurenine pathway".
- Spectral ad theoretical studies of KA.
- In S0 state, KA exists mainly in anionic form in water.
- The presence of keto tautomer in S1 state.

GRAPHICAL ABSTRACT

Existence of various species such as keto, enol, anion of Kynurenic acid, a well-recognized antiexcitotoxic and anticonvulsant drug, and the byproduct of tryptophan metabolism has been established based on the steady state and time resolved absorption and fluorescence spectroscopy. Quantum chemical calculations support the experimental findings.

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ABSTRACT

In the present contribution, we have explored ground and excited state spectroscopic properties of an antiexcitotoxic and anticonvulsant drug, Kynurenic acid (KA), through steady-state absorption, emission and time-resolved emission spectroscopy and quantum chemical calculations. The main focus of this article is to illustrate the effects of various parameters such as the nature of the solvents and pH of the medium on the spectral properties of KA which confirms the presence of different neutral and ionic species in the ground and excited states. The molecule KA exists mainly as keto- or anionic form in the ground state, whereas the main contribution of its emission is arising from the keto tautomer in the excited state. Quantum chemical calculations by Density Functional Theory (DFT) method has been effectively employed to correlate the experimental findings. The ground and excited state properties of KA ascertained by means of experimental and theoretical method reveal that it resembles well with other two compounds, 4-hydroxyquinoline and xanthurenic acid formed by the decomposition of UV filters.

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Introduction

Human lens and retina are protected from photodamage [1,2] by molecular UV filters consisting of low molecular weight compounds present in the lens. In fact, the UV radiation from solar light that finally reaches our eyes after transmission through the Earth’s sunscreen, i.e., the ozone layer, still contains ultraviolet components in the wavelength 300–400 nm, which may be detrimental to our eyes. But UV filters, present in the human lens, absorb UV light from 300 to 400 nm wavelength region, thereby protecting our eyes from UV-induced damage [1,3,4]. The well-known UV filters, namely kynurenine, 3-hydroxykynurenine and 3-hydroxykynurenine O-β-D-glucoside are originated as the product of enzymatic metabolism of amino acid, L-tryptophan [1,5–7]. These compounds can undergo spontaneous deamination [8] during photochemical, thermal and enzymatic reaction yielding highly reactive species which are capable to make important modification of lens protein, like nuclear cataract development [9–11]. The decomposition of UV filters under physiological conditions gives rise to the formation of different compounds. For example, thermal, photochemical or enzymatic reactions of kynurenine can produce 4-hydroxyquinoline (4HQN), 4,8-dihydroxyquinoline-2-carboxylic acid (xanthurenic acid, XA) and 4-hydroxyquinoline-2-carboxylic acid, commonly known as Kynurenic acid (KA). In 1853, KA was first identified when it was spotted in canine urine [12]. Half a century later, in 1904, the compound was recognized as a byproduct of tryptophan degradation [13]. KA is the end product in one of the branches of the “kynurenine pathway”. KA can be formed from kynurenine either through spontaneous deamination forming 4-(2-aminophenyl)-2,4-dioxobutanonic acid followed by cyclization, or under the action of an enzyme kynurenine aminotransferase [14,15]. KA is a renowned endogenous antagonist of the glutamate ionotropic excitatory amino acid receptors N-methyl-D-aspartate (NMDA) [16,17]. Till date, among different endogenous NMDA receptor antagonist, only KA can mediate glutamatergic hypofunction. Despite the NMDA receptor antagonism, KA can act as a nicotinic receptor antagonist [18]. The involvement of KA in the pathophysiology of psychiatric disorders, including schizophrenia, has been widely reported [19–21]. KA has not been found in normal lenses or its concentration is below the detectable level. Actually, KA is chemically or photodegraded too below the detectable level. KA has not been found in normal lenses or its concentration is below the detectable level. 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The steady state absorption and emission spectra of KA in different medium were acquired on Hitachi UV–Vis U-3501 spectrophotometer and PerkinElmer LS-55 fluorimeter, respectively, with quartz cuvettes of 1 cm path length. In all measurements, the sample concentration has been maintained within the range of 10⁻⁵–10⁻⁶ mol/dm³ in order to avoid aggregation and self quenching phenomena. All experiments were carried out at room temperature (298 K). ¹H nuclear magnetic resonance (¹H NMR) spectra were taken in d₆-DMSO using tetramethylsilane as an internal standard on a Bruker AV 3000 supercon spectrometer (300 MHz); chemical shift are in δ units (ppm). FT-IR measurements were carried out at room temperature using Perkin–Elmer Spectrum-100 spectrophotometer using the KBr wafer technique. The pH of different solutions was measured using pH system 361 pH meter from Systronics, India.

Fluorescence quantum yield (ΦF) of KA in different solvents has been achieved based on the secondary standard method using β-naphthol (ΦF = 0.23 in MCH) as the secondary standard and the following equation [27–29] has been used for this estimation:

\[
Φ_F = Φ_F^0 \frac{n^2}{n_0^2} \frac{OD}{OD^0} \frac{\int I_f(\lambda_f)d\lambda_f}{\int I_f(\lambda_f)d\lambda_f}
\]

Experimental section

Materials

Kynurenic acid (Scheme 1) was used as received from Sigma–Aldrich without further purification. Water was triply distilled for the preparation of aqueous solutions. Spectroscopic grade organic solvents methylcyclohexane (MCH), tetrahydrofuran (THF), 1,4-dioxane (DOX), chloroform (CHCl₃), acetonitrile (ACN), dichloromethane (DCM), dimethylsulfoxide (DMSO), isopropanol (iPrOH), 1-butanol (BuOH), ethanol (EtOH) and methanol (MeOH) were purchased from Spectrochem (India) and the purity of solvents have been verified by measuring their spectra in the wavelength range used. Sodium hydroxide (NaOH) and sulfuric acid (H₂SO₄) were obtained from Merck and triethylamine (TEA) from Spectrochem (India) was used as received. The surfactants sodium dodecysulphate (SDS), cetyltrimethylammoniumbromide (CTAB) and p-t-tert-octylphenoxo polyoxyethanol (Triton X-100, TX-100) were purchased from SRL, Spectrochem and Merck, respectively and used as received.

Instrumentations and methods

The present work is devoted to the exploration of the photophysical properties of KA in different solvents, pH etc. The important characteristics of various hydroquinolines, like 4HQN, XA, are that depending on the nature of the solvents, they can exist in the enol and keto tautomeric forms (E and K), and also undergo protonation and deprotonation reactions with the variation of the pH of the medium [22,23]. Similar to 4HQN and XA, KA can also exist as four different species, K, E, cation and anion, depending upon the acidity of the aqueous solution as reported by Pilieni et al. [24,25] and Zelentsova et al. [26]. Besides aqueous environment, up to now, the effect of different solvent–assisted tautomerization has not been taken under consideration. Therefore, for a better understanding of the ground and excited state properties of KA, we have employed electronic absorption and fluorescence spectroscopy to determine the energetically favorable forms of the target molecule in different solvents and with variation of pH of the medium. Effect of different surfactants on this molecule may also ascertain the most probable forms of KA in aqueous solution. So far our knowledge goes to, there are no detailed theoretical study on this molecule as was done for 4HQN and XA [22,23]. Quantum chemical calculations have been performed on the structural aspect of the molecule to correlate with experimental findings.

Scheme 1. Optimized structure of Kynurenic acid (KA) at DFT/B3LYP/6-31++G** level.
Where \( n_0 \) and \( n \) are the refractive indices of the solvents, \( OD \) and \( OD_0 \) are the optical densities, \( \phi_0 \) and \( \phi_f \) are the quantum yields and the integrals denote the (computed) area of the fluorescence bands. Each parameter is for the standard and sample solutions, respectively.

Fluorescence lifetime has been acquired using a Time Correlated Single Photon Counting (TCSPC) spectrophotometer (Horiba Jobin Yvon) employing a nanosecond diode laser (IBH, nanoLED-07) operating at \( \lambda_{ex} = 340 \) nm as the light source to trigger the fluorescence of KA in different medium at different emission maxima and TBX-04 as the detector. The emission signals from the samples have been collected with an emission polarizer kept at the magic angle (\( \sim 54.7^\circ \)). The decays have been evaluated using Data Station v-2.5 decay analysis software. The parameters which are considered here for goodness of fit are \( \chi^2 \) values, Durbin–Watson parameter and visually good residual plot. Average lifetimes (\( \tau_{avg} \)) of fluorescence for multiexponential decay have been calculated from the decay times (\( \tau \)) and pre-exponential factors (\( a \)) using the following equation:

\[
\tau_{avg} = \sum_i a_i \tau_i
\]  

Computational details

All theoretical calculations have been performed using Gaussian 09 software [30]. All possible ground state conformers like keto, enol, protonated, deprotonated form of KA (Scheme 2) have been optimized using Density Functional Theory (DFT) with B3LYP functional [31,32] and 6-31++G** basis set. \( ^1H \) NMR isotropic shielding \( \Delta \) values and **T** were calculated with the gauge including atomic orbital (GIAO) method using the optimized parameter obtained from B3LYP/6-31++G** method. The vibrational frequencies have been calculated with the same level of theory for the optimized structure and the obtained frequencies were scaled by 0.9781 [33]. In order to assign the aspects of different solvents on KA photophysics, theoretical calculation using Density Functional Theory–Polarized Continuum Model (DFT–PCM) have been executed. For excited state phenomenon, Time Dependent Density Functional Theory (TDDFT) has been applied with the same level of calculation as mentioned above.

Results and discussion

Absorption spectra

The UV–Visible absorption spectra of KA in different solvents have been pictured in Fig. 1a and the corresponding absorption maxima have been listed in Table 1. As shown in Fig. 1a, the absorption spectra in different solvents are structured and are similar to 4HQN [22]. The structured absorption band may be originated due to the presence of different species, enol (E), keto (K), anion (E\(^-\)/K\(^-\)) or zwitterions in solutions. In different organic solvents, excluding water, the absorption maximum is centered at \( \sim 345 \) nm with two shoulders at \( \sim 320 \) nm and \( \sim 360 \) nm, whereas in case of water, it is positioned at \( \sim 333 \) nm. Based on the previous literature, \( \sim 333 \) nm absorption band in aqueous medium has been assigned to be originated from the anionic form and in other organic solvents, the main contribution of the absorption band arises from the keto tautomer of KA. This assignment of absorption bands in different solvents has been further validated by quantum chemical calculation in the subsequent section. The effect of solvent mixture (DOX + H\(_2\)O) on the absorption spectra of KA can also endorse the presence of various tautomers in different solvents. Fig. 1b shows that with addition of water in DOX solution of KA, blue shifting occurs with the appearance of \( \sim 333 \) nm band and lowering the absorbance of \( \sim 345 \) nm band, thereby corroborating that in water, the major contribution in the absorption spectrum is arising from the anion (A\(^-\)) of KA. In DOX, the hump at \( \sim 360 \) nm vanishes in water. So, it can easily be concluded that in water, KA exists mainly in anionic form and as keto form in other solvents.

**Scheme 2.** Schematic presentation of different neutral and ionic species present in ground state in various pH medium.

**Fig. 1.** (a) Steady-state absorption spectra of KA (10–20 \( \mu \)M) in different solvents in room temperature (298 K). (b) Normalized absorption spectra of KA on the effect of solvent mixture (DOX + H\(_2\)O).
The pH variation experiments on the absorption spectral profiles of KA leads to some interesting modulations which indicates the presence of different species in aqueous solution under different circumstances.

In water, KA mainly exists in the anionic form (A−) exhibiting absorption band at ~333 nm. Incremental addition of acid to aqueous solution of KA leads to diminution of 333 nm absorption band with the emergence of new bands at ~311 nm and ~360 nm (Fig. 2a). The species formed on addition of acid which absorbs at ~311 nm can certainly be the protonated, i.e. the cationic form of KA (C+C). An isosbestic point at ~315 nm dictates equilibrium between the deprotonated and protonated form of KA. On the other hand, since KA exists as anionic form in water, addition of acid may facilitate the formation of neutral keto (K) form of KA absorbing at ~360 nm exhibiting another isosbestic point at ~357 nm. The structure of the absorption band at lower energy region is very much similar with the absorption band of KA in non-aqueous solvents. So, this can be attributed to the protonation of the acidic (–COOH) group with the formation of neutral K-form.

Effect of variation of pH

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In order to estimate the ground state acidity constant (pKₐ value) of KA in aqueous solution, the well recognized Weller’s titrimetric method has been applied in which the absorption intensities of the neutral and the conjugate acid (base) were plotted against the pH of the solution (Fig. 2b) [34,35]. Using this method, the ground state pKₐ values for the protonation of nitrogen atom and oxygen atom (–COOH) of KA is determined to be 2.87 and 2.99, respectively. Similar to the previous reports [24–26], it is difficult to predict which form of KA prefers to exist at low pH because protonation of carboxylic as well as pyridinyl group possibly be occurred at the same pH range.

The effect of addition of electron donor base, triethylamine (TEA), on the absorption spectra of KA in CHCl₃ solution is shown in Fig. 3a. Addition of base results in the prominent enhancement of the absorption band of KA in CHCl₃ exhibiting another isosbestic point at 311 nm and 333 nm, thereby indicating the presence of both mono- as well as di-anion of KA (Fig. 3b). The pKₐ value for the formation of the anion of KA, determined from the Weller’s titrimetric method (Fig. 3c), is 11.68. The pKₐ value is in good agreement with the previous report [24]. Excited state pKₐ value has also been calculated to be 7.22 using the following well known Förster–Weller equation [36].

\[
pK'_{a,e} = pK'_{a} + \frac{0.625}{T} (\nu_{\text{neutral}} - \nu_{\text{anion}})
\]

where \(\nu_{\text{neutral}}\) and \(\nu_{\text{anion}}\) are the energies (in wavenumber) of the (0, 0) transition of neutral and anion, respectively, and \(T\) is temperature in Kelvin. The lowering of pKₐ value in the excited state points towards the fact that KA becomes neutral in the S₁ state, exhibiting only keto emission. Similar to the deprotonation phenomenon, excited state pKₐ value can also be determined from the protonation episode and the corresponding value of pKₐ is 7.71. This enhancement of pKₐ value also endorses the presence of keto tautomer in the excited state.

Emission and excitation spectra

Fig. 3a depicts the steady state fluorescence emission spectra of KA in solvents of varying polarity and the observed band maxima are presented in Table 1. The intensity of KA fluorescence is found to be strongly solvent dependent. In non-polar solvents, like MCH, DOX, KA is practically non-fluorescent, while in polar protic solvents such as in MeOH and H₂O, it shows high fluorescence intensity. Irrespective of the nature of the solvents, single fluorescence band of KA emerges at ~390 nm and the position of emission maxima does not change with the variation of the excitation wavelength (Fig. S1). Since, KA is structurally similar to 4HQN and 4HQN exists only in the keto form in aqueous solution [22], so it can be easily presumed that the keto form is the major tautomeric state of KA contributing to the emission at ~390 nm. Additionally, the excitation spectra (Fig. 4b) of KA in different solvents further authenticates the presence of single tautomeric form in the excited state. As shown in Fig. 4b, the fluorescence excitation spectra in all solvents at their relevant emission band positions are found to be

### Table 1

Spectroscopic parameters of KA obtained from UV-Vis absorption and emission spectra in different solvents at room temperature.

<table>
<thead>
<tr>
<th>Solvents</th>
<th>(\lambda_{\text{abs}}) (nm)</th>
<th>(\lambda_{\text{emi}}) (nm)</th>
<th>(\Phi_{f})</th>
</tr>
</thead>
<tbody>
<tr>
<td>DOX</td>
<td>334, 346, 360</td>
<td>398</td>
<td>0.0035</td>
</tr>
<tr>
<td>CHCl₃</td>
<td>334, 346, 361</td>
<td>398</td>
<td>0.0117</td>
</tr>
<tr>
<td>+TEA</td>
<td>322, 335, 350</td>
<td>378</td>
<td>-</td>
</tr>
<tr>
<td>ACN</td>
<td>330, 344, 357</td>
<td>398</td>
<td>0.0112</td>
</tr>
<tr>
<td>DMSO</td>
<td>322, 332, 346, 360</td>
<td>401</td>
<td>0.010</td>
</tr>
<tr>
<td>PrOH</td>
<td>320, 332, 345, 358</td>
<td>394</td>
<td>0.0137</td>
</tr>
<tr>
<td>BuOH</td>
<td>320, 332, 346, 361</td>
<td>382</td>
<td>0.0182</td>
</tr>
<tr>
<td>MeOH</td>
<td>318, 331, 343, 357</td>
<td>380</td>
<td>0.0425</td>
</tr>
<tr>
<td>H₂O</td>
<td>321, 333, 345</td>
<td>386</td>
<td>0.085</td>
</tr>
<tr>
<td>+H⁺</td>
<td>311, 329, 365</td>
<td>425, 460</td>
<td>-</td>
</tr>
<tr>
<td>+OH⁻</td>
<td>319, 326</td>
<td>398</td>
<td>-</td>
</tr>
</tbody>
</table>
reasonably similar to the absorption spectra. Thus, it can be concluded that the emission spectra of KA in different solvents (except water) arise from the K form. On the contrary, in case of water, the emission spectrum arises from the ground state anion of KA.

Solvent dependency of KA fluorescence intensity is further ascertained by fluorescence quantum yield ($\Phi_F$) values obtained by using Eq. (1). As clear from Table 1, in non-polar solvent DOX, KA has very low quantum yield value, whereas in polar protic solvent, $\Phi_F$ value increases with increase of hydrogen-bonding ability of the solvent. Fig. S2 certifies that in protic solvents, there is an evident of correlation between hydrogen-bonding parameter ($\alpha$) of the solvent and $\Phi_F$ values of KA in protic solvents.

Effect of medium pH on fluorescence emission spectra

As in the case of the absorption spectra, variation of pH has a pronounced effect on the fluorescence spectral properties of KA. Fig. 5a dictates the changes in fluorescence emission spectra of KA with incremental addition of acid in aqueous medium. Red shift of emission maxima from $\sim$386 nm to $\sim$425 nm along with intensity diminution is observed with decrease of pH of the aqueous solution of KA. Excitation spectra monitored at $\sim$425 nm emission band in acidic medium, shown in inset of Fig. 5a, matches perfectly well with the absorption spectra of KA in aqueous solution in presence of acid (Fig. 2a). Therefore, the formation of cation of KA as a result of action of the acid (Scheme 2) is in excellent agreement with the aforementioned observation. Hence this band ($\sim$425 nm emission band) has been assigned to emission from the cationic form of KA.

Addition of dilute NaOH in an aqueous solution of KA is displayed in Fig. 5b. With increasing the pH of the medium, the emission wavelength experiences a red shift ($\sim$386 nm to $\sim$398 nm) with concomitant decrease in fluorescence intensity. Modifications of excitation spectra of KA in presence of base from that in absence of base, monitored at 398 nm (inset of Fig. 5b), reflects similar pattern as was observed in the absorption profile (Fig. 3b). Thus, this $\sim$398 nm emission band can be formally attributed to the anionic species formed upon deprotonation of –COOH group of KA. As evident from Figs. 5a and b, with variation of pH, fluorescence of KA quenches, but protonation of quinoline nitrogen atom results stronger fluorescence quenching (83%) than that of deprotonation phenomenon (48%), as depicted in Figs. 5a and b.
Fluorescence lifetime measurements can provide useful information about the excited state of a molecule. In order to achieve deeper insight into the photophysics of KA, we have employed TCSPC measurements. The fluorescence time profiles of KA in different solvents as well as for acidic, neutral and basic aqueous solutions have been recorded and the obtained data are plotted and listed in Fig. 6 and Table 2, respectively. In most of the cases, the emission profiles exhibit biexponential decays in order to obtain reasonable values and best residual plot (Fig. S3). The observed biexponential decay pattern of KA consisted of a major fast component and a minor slow component. For example, the values obtained in water medium are \( a_1 = 0.997, \quad s_1 = 0.128 \text{ ns}, \quad a_2 = 0.003, \quad s_2 = 0.802 \text{ ns} \) and \( \chi^2 = 1.00 \). The major component with ultrafast decay in all solvents has been assigned to the K-form of the molecule. On protonation or deprotonation event, excited state lifetime value increases as evident from Table 2. With addition of acid, \( s_1 \) value of the protonated conformer goes to 0.243 ns (99.7%) and with addition of base, NaOH, \( s_1 \) value becomes 0.140 ns (99.7%) for the deprotonated form of KA. This time resolved study indicates that ultrafast keto form would be the most populated conformer on the excited \( S_1 \) surface and this result is in accord with the previous study [26].

### Effect of surfactants on KA

Here, we have investigated the effect of cationic, anionic and neutral surfactants on the emission as well as excitation spectral properties of KA in aqueous medium. Fig. 7a depicts the series of emission spectra of KA in absence and presence of different concentration of cationic surfactant, cetyltrimethylammonium bromide (CTAB) and the corresponding excitation spectra has been portrayed in inset of Fig. 7a. The fluorescence decay pattern of KA in water and CTAB solution is shown in Fig. 7b. Since, with the addition of different amounts of CTAB, the decay fitting is tri-exponential in nature, as shown in Table 2, we have considered average lifetime value (\( \tau_{avg} \)) in order to get rid of more complexation. The \( \tau_{avg} \) value becomes longer (0.193 ns) than water (0.130 ns) indicating the
residence of the dye in a protective restricted environment of the micellar hydrophobic core. We perform the same experiment with an anionic surfactant, sodiumdodecyl sulfate (SDS) and a non-ionic surfactant, Triton-X (TX-100) and found no noticeable change in the emission spectral properties of KA. This indicates that negatively charged KA does not interact with anionic SDS and neutral TX-100 surfactants.

**Theoretical calculations**

In order to correlate our experimental findings, we have performed computational calculations of KA molecule in the electronic ground state at DFT level using B3LYP functional and 6-31++G** basis set and in the excited singlet state at TDDFT level using same level of calculations. Out of all probable low energy conformers of KA, E- and K-form can be predicted to be two stable conformers in the ground state. Between these two forms, which form would be the most stable can be determined by their energy difference. In water, K- form (−0.63 kcal/mol) is much more stable than E- form (0.0 kcal/mol), so we can discard the presence of enol form in the ground state. We have also evaluated relative Gibbs free energies (∆G_{K,E}) of the enol and keto tautomers in the gas phase as well as in a series of solvents using the PCM model. The corresponding values are 0.96, −0.89, −0.89 and −0.89 kcal/mol in the gas phase, ACN, DMSO and water solutions, respectively.

These theoretical results obviously predict that keto form is found to be energetically most favorable in most of the solvents. The calculated S0→S1 transition energy for keto form in different solvents obtained from the TDDFT-PCM calculations are listed in Table 3. As seen in Table 3, the experimental and theoretical values for absorption energies match reasonably well and hence our prediction is supportive to experimental results.

Fig. 8a shows the comparative picture of the 1H NMR spectrum of KA experimentally and theoretically. Experimentally, the characteristic peaks, shown in the spectra (Fig. 8a1), namely H3, H6, H7, H8 and H9 are observed at 6.552, 7.991, 7.278, 7.856 and 7.608 ppm, respectively. Conversely, NMR analysis on the optimized molecular geometry of KA (K- form) at B3LYP/6-31++G** level, as shown in Fig. 8a2, assigns the signal as follows: H3, 6.375 ppm; H6: 8.038 ppm; H7: 7.611 ppm; H8: 8.038 ppm; H9: 7.788 ppm. Experimentally determined NMR spectra simulates well with the theoretically computed NMR spectra of K-tautomer of KA. So this method of comparison may also be a supporting evidence for the existence of K-tautomer.

Similarly, infrared spectroscopy may be a helpful tool for investigating the existence of keto/enol tautomer in ground state. To gain a better understanding of the photophysics of KA, FT-IR spectroscopy has been applied here, theoretically and experimentally. As shown in Fig. 8b1, the appearance of band at ~3480 cm⁻¹ attributed to N-H stretching frequency is good evidence for the assignment of the compound to be keto one, since O–H stretching frequency of 4-hydroxypyridine occurs at 3640 cm⁻¹ and the IR spectrum of 2-pyridone shows a strong absorption band of N–H group at 3450 cm⁻¹ [37]. On the contrary, IR spectrum obtained by frequency test on the optimized structure of the keto form of KA is displayed in Fig. 8b2. This theoretically evaluated plot matches well with the previously discussed experimental plot (Fig. 8b1), as N–H stretching frequency arises at ~3577 cm⁻¹. So, NMR as well as IR results are favor of the dominance of the keto form in neutral solution.

On the basis of vibrational investigation at DFT/B3LYP/6-31++G** level, the standard statistical thermodynamic parameters, like total thermal energy (E), standard heat capacities (Cv), standard entropies (S), and standard enthalpy changes (∆H0→T) for the studied compound, KA, in gaseous phase, have been evaluated as a function of wide temperature range and pictured in Fig. S4. From Fig. S4, it can be observed that the standard E, Cv, S and ∆H0→T are increasing with temperature ranging from 100 K to 800 K. This is because when the temperature is lower the main contributions to the thermodynamic functions are from the translations and rotations of molecules, while the vibrational movement is intensified at higher temperature and makes more contributions to the thermodynamic functions. Fig. S5 also endorses the above statement as vibrational component of total entropy rapidly changes with temperature while that of translational and rotational part of entropy changes minutely. The correlation equations between these thermodynamic parameters and temperatures have been fitted by quadratic formulas and the corresponding fitting equations with their respective fitting factors (R²) are listed below.

\[
E = 96.01352 + 0.01212 \times T + 5.08185 \times 10^{-5} \times T^2; R^2 = 0.99964
\]  

(4)

**Table 3**

<table>
<thead>
<tr>
<th>Solvents</th>
<th>Experiment</th>
<th>Calculation</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>S0 (nm)</td>
<td>Keto (nm)</td>
</tr>
<tr>
<td>DMSO</td>
<td>322, 332, 346, 360</td>
<td>357</td>
</tr>
<tr>
<td>ACN</td>
<td>330, 344, 357</td>
<td>356</td>
</tr>
</tbody>
</table>
In the present article, the detailed photophysical investigation of a biological photosensitizer, Kynurenic acid (KA), has been carried out by experimental and theoretical perspective on the basis of steady-state absorption and emission, time resolved emission spectroscopy and quantum chemical calculations. Spectroscopic signatures on KA by the variation of medium polarity and variation of medium pH enable us to assess the presence of different absorbing and emitting species under different experimental conditions. In aqueous solution, KA mainly exists in anionic form (~333 nm) in the ground state, but in other solvents it exists in the keto form. On the contrary, on excitation, the main contribution of its emission (~386 nm) is coming from its keto form. The increase of acidity (pK_a), in excited state, substantiates single emission of KA, irrespective of the nature of the solvents. Anionic nature of KA is further ascertained by exploring the effect of cationic surfactants, CTAB, whereas no significant spectral change is observed in presence of anionic surfactant, SDS and neutral surfactant, TX-100. Computational evaluations based on DFT/B3LYP/6-31++G** method proves to be an efficient tool to correlate the experimental findings. Structural optimization shows that K-form is the most stable form in most of the solvents. Both the theoretical and experimental 1H NMR as well as IR spectroscopic results are in favor of the existence of the keto form in ground state. Theoretically predicted electronic absorption maximum of KA are in a good agreement with experimental data. In conclusive remarks, the ground and excited state properties of this biologically significant molecule with the variation of solvents nature and pH inferred from the experimental data are well complemented from computational calculations and this detailed photophysics will provide helpful information to further study of its future applications.

Conclusion

All the thermodynamic data provide helpful information to further study on the title compound in field of thermochemistry.

Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.jsaa.2014.03.079.

References
