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13.1 Introduction

The oxidation processes and the underlying chemical reactions that occur in organisms can generate molecules with electronically excited states that can emit light (Cilento 1973; Slawinska and Slawinski 1983; Vacher et al. 2018; Pospíšil et al. 2019; Cifra and Pospíšil 2014; Saeidfirozeh et al. 2018a). There is evidence that singlet oxygen (Miyamoto et al. 2007; Miyamoto et al. 2014) and triplet excited carbonyls (Vacher et al. 2018; Cilento and Adam 1995) are the two main excited state molecules that are primarily generated via chemiexcitation in biological systems. Although singlet oxygen emission spectra have been well-explored both experimentally (Schweitzer and Schmidt 2003; Adam et al. 2005; Losev et al. 1988) and theoretically (Schweitzer and Schmidt 2003; Minaev et al. 2009; Minaev 2017), there is no data on theoretical molecular-level calculations on emission from triplet excited carbonyls. The main physical parameter of interest that can be directly compared with experimental data is the emission spectrum of excited triplet carbonyls. Aldehydes are the most frequently analyzed carbonyls within this research field. Experimental evidence for electron-excited aldehydes generated through chemiexcitation suggests that the peak of the emission is in the range of 400–450 nm (Bechara et al. 1979; Cilento 1984; Escobar et al. 1990). Spectra obtained by measuring the phosphorescence of aldehydes (excited states obtained by external light excitation) have confirmed this range (Oliveira et al. 1978; Schuh et al. 1984). There is also very extensive experimental evidence that carbonyls are generated during oxidative reactions – in fact, carbonylation is one of the well-accepted and widely used markers of oxidation of biomolecules (Rogowska-Wrzesinska et al. 2014; Purdel et al. 2014; Yan and Forster 2011). However, chemical detection of carbonyls does not provide information if the carbonyls were produced in an excited or ground state. Quantum chemical modeling of one of the processes (dioxetane decomposition), which is believed to generate excited state carbonyls, provides additional evidence that most of the excited state carbonyls are generated through chemiexcitation are in the triplet excited state (Farahani et al. 2013; Augusto et al. 2017). The triplet excited carbonyls may then undergo various decay pathways, including further reactions, transferring electron excitation energy to acceptors (Bohne et al. 1986; Durán and Cilento 1980) (which can then emit energy at longer wavelengths) or directly emitting the photon.

Molecular oxygen (O_2) is a known quencher of excited state carbonyls. Quenching takes place by excitation energy transfer from a carbonyl to O_2 and creating O_2 in an excited state (singlet oxygen, $^1O_2$) (Bastos et al. 2017; Gundermann and McCapra 1987). Various energy substrates of $^1O_2$ have also been claimed to be able to emit ($^1O_2$ dimolar emission) at wavelengths that are (Khan and Kasha 1970) close to that of the carbonyl triplet excited state, hence creating ambiguity in the spectral evidence of carbonyl emission. Therefore, it is important to obtain a deeper theoretical understanding of the emission spectra of the carbonyl triplet excited state. Quantum mechanical modeling approaches are known to provide accurate predictions of the transitions between vibrational and electronic energy levels in molecules. Such an approach can be used to model the absorption and emission of photons

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by molecules. Following our preliminary work (Saeidfirozeh et al. 2017; Saeidfirozeh et al. 2018b), the emission from the triplet excited states has been modeled in the case of the simplest molecules that contain carbonyl groups: formaldehyde, acetaldehyde, and acetone.

13.2 Chemiluminescence from Biological Systems as a Triplet Excited State Indicator

The lowest energy singlet excited state can undergo spin-orbit coupling (SOC), an intersystem crossing process into an energy state of higher spin multiplicity, which is known as a triplet state. The triplet state has a low transition probability (long decay time) to the ground state through the 'dipole forbidden' process because of the spin mismatch between the triplet and the singlet ground states. If it does not lose energy through non-radiative channels, it can decay radiatively to the singlet ground state with a 'dipole forbidden' but spin-orbit (SO) allowed transition. These transitions progress with significantly slower time scales because of the weakness of the SO interaction. If the quantum yield of these processes is large, it will release significant amounts of light over long time scales and create the so-called glow-in-the-dark effect.

As shown in Fig. 13.1, eventually, a chemical process can engender a new molecule in an excited state. If the system does not undergo a vibrational relaxation to the singlet ground state, a possible SOC interaction can allow a spin flip, moving it to the manifold of the triplet excited states. Within this manifold, because 'dipole allowed transition' is forbidden between states of different multiplicity, the system can perform further vibrational relaxation to the lowest energy triplet state, where it can reside for longer (on a molecular scale and compared with spin-allowed transitions) time. SOC can then allow a radiative process to S_0, giving rise to photon emission. Thus, this phenomenon can be expressed as:

$$\text{Chemical reaction} \rightarrow S_1 \rightarrow T_2 \rightarrow T_1 \rightarrow S_0 + h\nu_1 \quad (13.1)$$
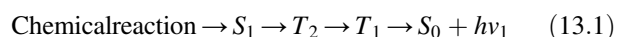
in which S_0 and S_1 are the singlet ground state and singlet excited state, respectively, T_1 and T_2 are the triplet excited states, and $h\nu_1$ is the vertical lowest energy. To calculate the correct energy related to the emission from the triplet state, first, the geometry of the molecule in both the ground and excited state needs to be optimized.

The carbonyls we selected to analyze (formaldehyde, acetaldehyde, and acetone) have different geometry in the ground and excited state. These molecules have a planar geometry in their singlet ground state, in which all the atoms are in the same plane (See S_0 energy level in Fig. 13.1).

However, in the first triplet excited state, these molecules show non-planar pyramidal geometry, in which they are bent with a τ (H-C-O-H) angle, (H-C-O-C) angle, and (C-C-O-C) angle in formaldehyde, acetaldehyde, and acetone, respectively (bent by approximately 35° compared with their planar configuration) (See T_1 energy level in Fig. 13.1). It is known that in these molecules, the $n \rightarrow \pi^*$ state is pyramidally distorted in the triplet excited state (Hadad et al. 1993). For more details about the electronic structure of these molecules, an interested reader is referred to (Demtröder 2010).

13.3 Computational Approach

All first principle calculations were performed with the Gaussian 16 Rev. B.01 program package (Frisch et al. 2016). Moreover, the wavefunctions were expanded with the 6311+g(d,p) triple-zeta valence Gaussian-type basis set with polarization and diffuse functions (Krishnan et al. 1980).

First, the geometry of the molecules in both the ground and the triplet excited state was optimized. Then the vibrational frequencies for these optimized geometries in both states were analyzed to confirm that all positive frequencies were obtained. Afterwards, the emission from the triplet excited state to the singlet ground state was calculated. Optimized geometries for both states and emissions' spectra were calculated by using twelve methods (See Fig. 13.2) to determine how different levels of theory and basis set affect the results. Because the emission of these molecules has also been studied experimentally (Robinson and DiGiorgio 1958; Charlesby and Partridge 1965; Mano et al. 2014), we could make a comparison with our theoretical results.

As shown in Fig. 13.2, it is clear that the Møller-Plesset perturbation theory at second order (MP2) method for these three molecules has the best agreement with experiments. This method outperforms even the best available density functional theory (DFT) calculation (Goerigk and Grimme 2011).

Because water is the common environment in which these excited states are generated, we investigated the effect of water solvents on the emission spectra.

However, it is very difficult to study the effect of discrete water molecules on the photo-physical properties owing to the many possible water arrangements around the studied molecules, whereas we are interested in their average effect on these properties. To avoid any complexity owing to the interaction between water and our molecules, we used the implicit solvation model. To address this important aspect, we employed the Polarizable Continuum Model (PCM), which uses the integral equation formalism variant (IEFPCM). This method creates the solute cavity by using a set of overlapping spheres surrounding the molecule (Tomasi et al. 2005). The procedure uses a set of spheres centered on
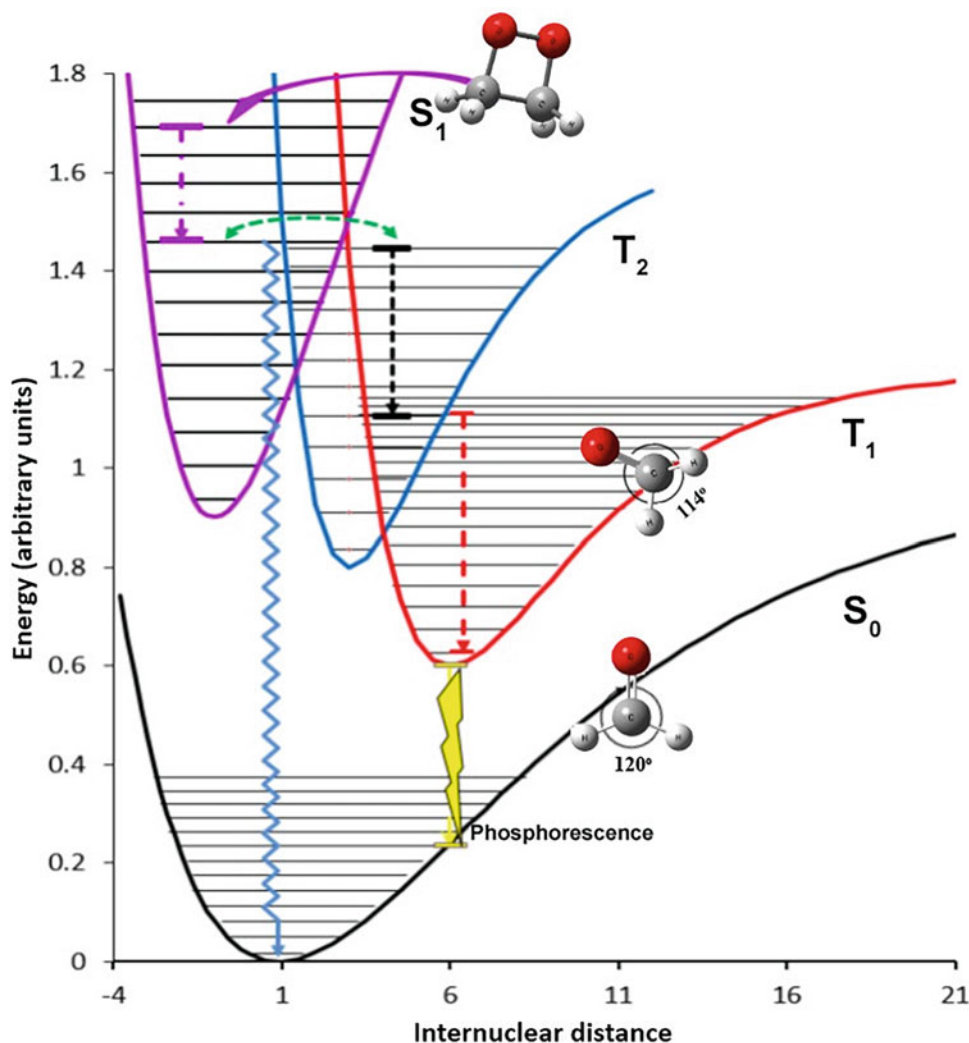
Fig. 13.1 Sketch of the possible Jablonsky-like energy plot of the adiabatic potential energy surface. S_0 and S_1 represent the singlet ground state and singlet excited state (black and purple lines), respectively. T_1 and T_2 represent the first and second triplet excited states (blue and red lines). T_2 could be assigned as any possible higher triplet excited state (Howarth et al. 2015). Abscissas-parallel thin lines within the Morse-like plots represent vibrational states associated with each adiabatic potential energy surface. The heavy purple arrow represents a possible chemical process (e.g., dioxetane decomposition) that engenders a carbonyl group in the S_1 state in some of its excited vibrational states. Dashed arrow lines represent vibrational cascades within each excited state. The double arrow green dashed line represents the intersystem crossing process enabled by SOC between states of different multiplicity. Further internal conversion within T_2 and interstate transition to T_1 allow the system to reach a state of lower energy. The radiative transition from T_1 to S_0 (yellow 'bolt-like' line) is enabled owing to SOC between T_1 and S_0. This radiative transition is the one that is suggested to give rise to photon emission from triplet excited carbonyls in endogenous chemiluminescence. A further non-radiative decay channel from S_1 is represented by the blue zig-zag line. See (Duben et al. 2013; Vo-Dinh and Cullum 2014; McLaren and Shugar 2016) for more information about excited states manifolds and transitions in general and (Ball 2014) for aldehydes, including the discussion of higher triplet states, such as T_2
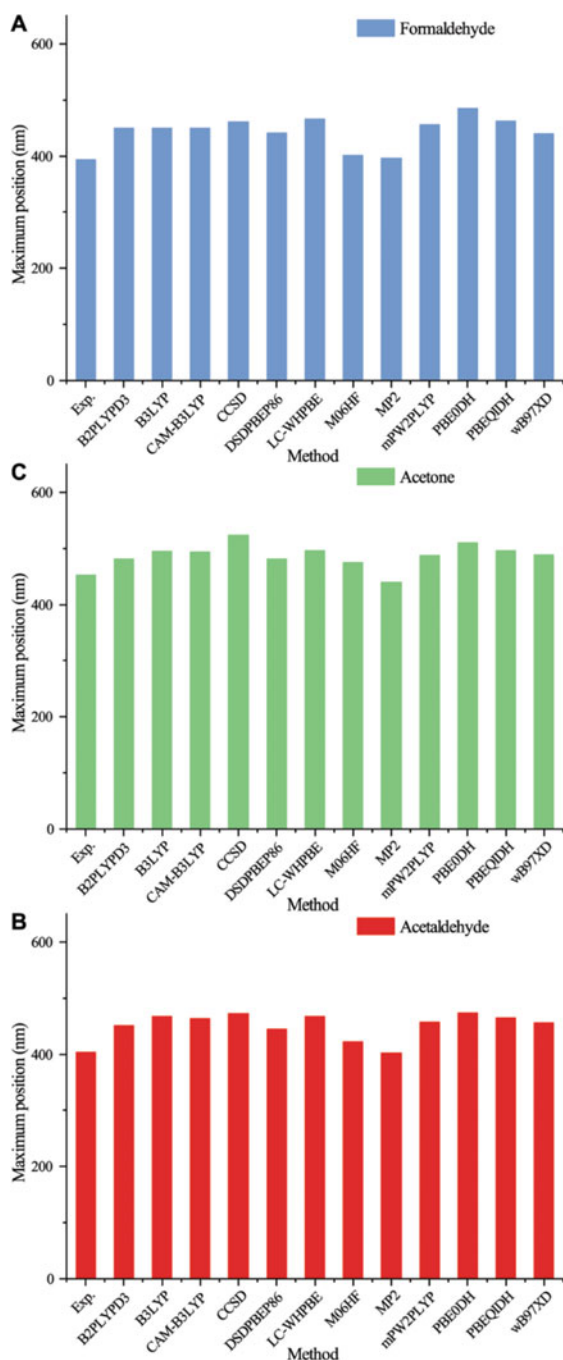
Fig. 13.2 The wavelength of the maximum (peak) in the water environment of the triplet emission spectra of the studied molecules for the different levels of theory used for its computation. The abbreviations of the theoretical approaches are labeled on the X-axis. The blue, red, and green bar charts refer to the peaks for formaldehyde, acetaldehyde, and acetone, respectively

each molecule atom and the inner surface defines the place where the reacting field of the solvent is computed. The

results of the calculated T_1-S_0 emission spectra are shown in Fig. 13.3. The emission spectrum of the molecules in water is consistently blue-shifted compared with the gas phase for each of the three carbonyls. This finding might have relevance in biological systems: in enzymatic systems, it has been suggested that the excited state is generated from the substrate in the cavity of the enzyme (Baader et al. 1985). The static dielectric permittivity (which affects the polarizability) of the protein ($\epsilon_0 = 2$–3) (Zhao et al. 2011; Krivosudský et al. 2017) is typically much closer to the vacuum value than the static permittivity of water ($\epsilon_0 = 80$) (Ellison 2007; Cifra et al. 2019).
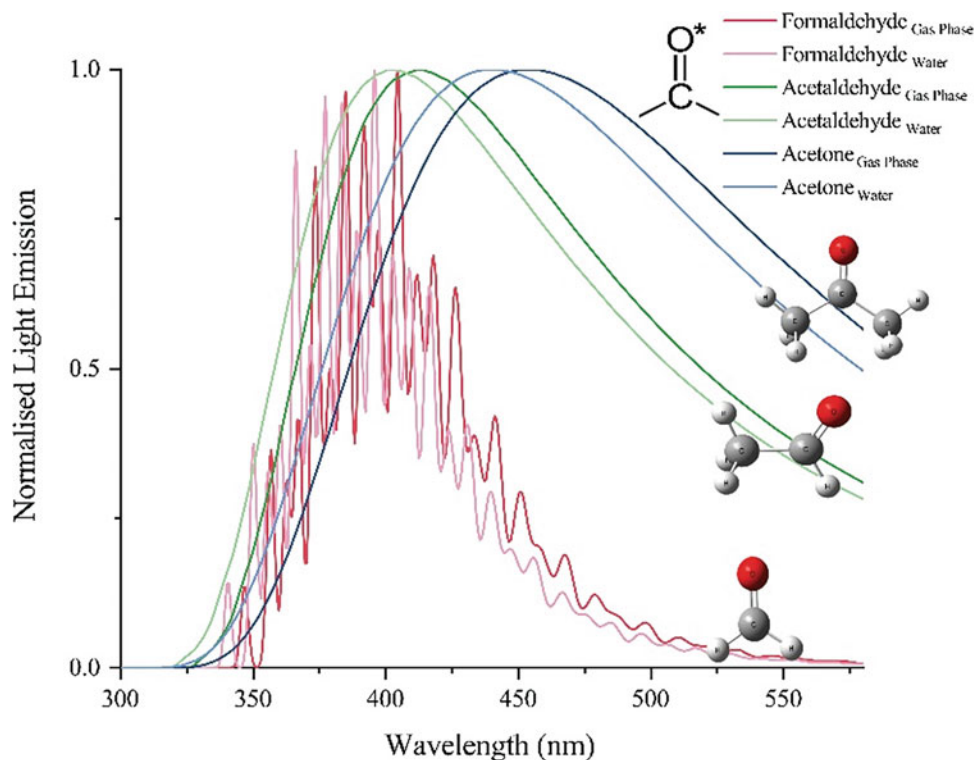
Furthermore, these results also showed that the presence of further methyl groups causes a shift of the spectra to longer wavelengths. Here, we should highlight that, in the case of formaldehyde, because the manifold of vibrational states is not as dense as in the case of larger molecules, the pattern of transitions between vibrational states belonging to the excited T_1 and the S_0 ground state is evident. In the case of acetaldehyde and acetone, the higher density of the vibrational states in T_1 and S_0 smooths out the plot, overriding the vibronic details. In general, our theoretical results match the experimental values obtained for the peak of the chemiluminescence in the blue region (400–450 nm), which is presumed to come from tripled excited carbonyls.

13.4 Conclusion

In this chapter, we demonstrated how modern quantum chemical approaches can provide a deeper understanding of the detected spectrum of biological chemiluminescence. We foresee that a massive application and computational screening of the spectra of various carbonyl species (as potential emitters) can potentially enable mechanistic interpretation of shifts of the emission spectra during various physiological states, diseases, or from various biological species. Such mechanistic understanding will foster the use of endogenous biological chemiluminescence in analytical, biotechnological, and biomedical applications.

Fig. 13.3 Theoretical spectrum of T_1-S_0 phosphorescence emission of formaldehyde, acetaldehyde, and acetone in the gas phase and water environments. Vibrational effects on the spectrum are visible in the case of formaldehyde (see text for more details)

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