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Experimental and theoretical study of the spectral behavior of Trypan Blue in various solvents

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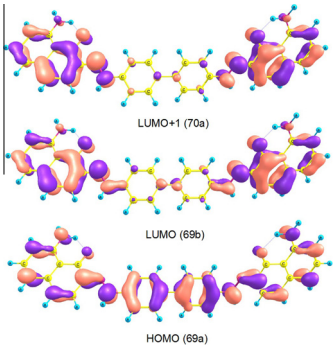
HIGHLIGHTS

- The electronic absorption spectrum of Trypan Blue was found to be dependent on solvent polarity.
- No enol tautomer was observed in any of the solvents studied.
- Existence of the keto only form is supported by NMR, IR and DFT calculations.
- The two visible absorptions are assigned to two different transitions in the keto form.
- Relationships between absorption wavelength and solvent parameters are examined.

GRAPHICAL ABSTRACT



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ABSTRACT

The absorption spectra of Trypan Blue were recorded in various solvents. The absorption maxima of the dye were found to be dependent on solvent polarity. NMR and IR data suggest that dye exists in the keto form in all solvents studied. Aggregation of Trypan Blue was observed at low pH values. The electronic absorption spectra of Trypan Blue in various solvents were also analyzed in terms of absorption wavelength and the refractive index value given as a function of $f(n^2)$ and a linear relationship was found. In a mixture of binary solvents (water and DMSO), the dye molecule tends to form a hydrogen-bonding solvated complex with the more polar solvent. The value of molecular complex formation constant (K_f) for the dye/DMSO complex and the Gibbs free energy (ΔG) were calculated. Density functional calculations indicate significant enhancement in internal hydrogen bonding for the keto form, resulting in a large energy difference between the keto and enol forms, consistent with the experimental observation of the existence of the keto form only. Time dependent density functional theory calculations suggest that the two visible absorption maxima observed experimentally arise from a single species in solution. The lower energy band is assigned to the HOMO → LUMO transition, and the second band to the HOMO → LUMO + 1 transition.

1. Introduction

The interactions between the various solvents and a probe molecule comprise a challenging area of study in solution chemistry. Changes in the intensity, position and shape of the absorption spectra can be used to understand the specific and nonspecific interactions between the probe and solvent molecules. These include aggregation, tautomerism, and hydrogen bonding, as well as electrostatic, hydrophobic and acid/base interactions. Each type of interaction is dependent on the environment. Solvent–solute interaction and micro-structuring are some of the features which have been investigated for several compounds such as azo dyes [1–4]. The azo dyes have drawn attention in this regard because

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of their photosensitivity and the bonding ability of substituents to N=N groups. Keto-hydrazone/enol-azo tautomerism in azo compounds is quite interesting both from theoretical and practical point of view and has been reported in various solvents [5–9]. The presence of tautomerism in these compounds is strongly influenced by several factors including temperature, substituents structure and solvent polarity [10–12]. Keto-hydrazone/enol-azotautomerism has been studied by various optical techniques. The tautomers of azodyes can be easily detected from the absorption spectra in the visible region. The aim of the present study is to investigate the solute–solvent interaction of Trypan Blue (a diazo dye) and its possible tautomeric forms in various solvents.

2. Experimental

Trypan Blue (C.I. name Direct Blue 14) was obtained from Clariant Chemicals and was used as such. Solvents used in this work were water, methanol, ethanol, dimethylsulfoxide (DMSO), dimethylformamide (DMF), ethylene glycol, 2-methoxyethanol and ethanolamine, which were procured from Merck and were of spectroscopic grade. All these solvents were stored over 4 Å molecular sieves throughout the course of the experiments. No other method was employed to purify these solvents. The spectral studies were done at room temperature (23 ± 2 °C) in UV and visible regions, with appropriate UV cut-offs, for 12 μM solutions. This concentration lies within the linearity range of Beer–Lambert law. Absorption spectra of dye solutions were recorded on a CARY 50 spectrophotometer, using a 10 mm quartz cell. Samples for IR spectra were prepared by mixing Trypan Blue with the various solvents to produce mulls. IR spectra were recorded on a Nicolet Impact 410 FTIR instrument using 32 scans and background correction. IR spectra were recorded in the range 800–4000 cm^{-1}. The NMR spectra were recorded in D_2O and DMSO-d_6 on a Varian 400 MHz NMR spectrometer.

3. Computational methods

All calculations were performed using the Gaussian 09W C01 program [13]. Model complexes 1 and 2 were derived by replacing sulfonate and methyl groups in the keto and enol forms of Trypan Blue with hydrogen atoms (Fig. 9). Geometry optimizations for the model complexes were performed in the gas-phase using the B3LYP hybrid functional and 6-311 + G(d,p) basis set. Geometry optimization calculations for the full keto and enol structures were performed with the same method and basis, but using the PCM solvation model [14,15] with water as the solvent. Solvent was included in all calculations on the full complexes as the 4^- charge of the salt form leads to positive eigenvalues for occupied orbitals in the gas phase. All electronic spectra were calculated with the CAM-B3LYP functional [16] and 6-311 + G(d,p) basis: The spectra of the model complexes were calculated both in the gas phase and with the PCM model using water as the solvent. The spectra of the full complexes were calculated using the PCM solvation model with water as the solvent. All calculated optimized structures were verified to be energy minima using vibrational frequency calculations. No symmetry constraints were used in geometry optimizations, but all optimized geometries were found to adopt C_2 symmetry. For TDDFT calculations, the first 12 allowed excitations were determined under C_2 symmetry.

4. Results and discussion

The structure of Trypan Blue is shown in Fig. 1. The present study deals with the spectral changes of this compound in different solvents. Absorption spectra were recorded in different solvents with the aim of understanding the solute–solvent interactions. The electronic absorption spectra of Trypan Blue were recorded in various solvents at room temperature, in the 200–800 nm range and are shown in Fig. 2 and tabulated in Table 1.

Trypan Blue dye can potentially exist in two tautomeric forms as shown in Fig. 1. Such equilibria have been studied for other structurally related dye molecules [17]. In the present study it was found that the dye exists only in the keto form for all solvents studied. This was evident from our IR studies of the dye in various solvents and was also confirmed by ^{13}C NMR studies in deuterated solvents (water and DMSO). The NMR spectrum in DMSO is given in Fig. 3.

4.1. Solvent effect on absorption spectra

The change in peak position of the absorption band in different solvents is an indication that the absorption wavelengths are

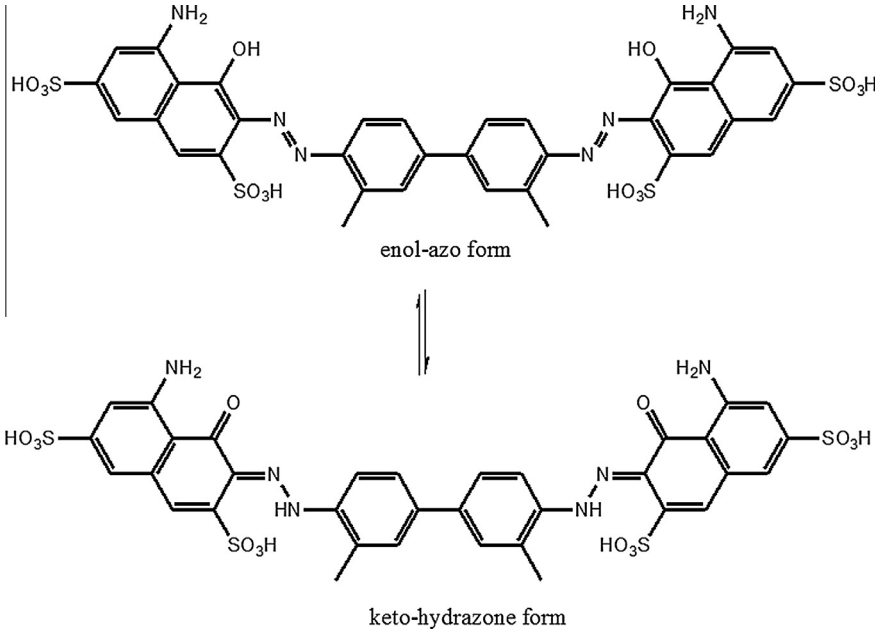

Fig. 1. Structure of Trypan Blue shown in enol-azo and keto-hydrazone forms.