# Isosbestic points and vibrational levels in electronically excited states of dye molecules

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## Abstract

The appearance of isosbestic points in the temperature-dependent electronic absorption spectra of a single type of molecule in solution can be due to the temperature dependence of the extinction coefficients caused by the Boltzmann distribution. We show that in this case the isosbestic points can be attributed exactly to vibrational levels of the excited electronic state. Isosbestic points are observed in the temperature-dependent spectra of rhodamine-3B-perchlorate and pyronine-101-perchlorate and can be attributed to the vibrational levels of the $S_1$ state.

## 1. Introduction

The presence of isosbestic points in a set of spectra, generated by changing the composition or temperature, can be used under appropriate circumstances as evidence for the number of independent reaction parameters [1–5]. However, isosbestic points are also observed if a single type of molecule in solution is studied under varying external parameters such as temperature, pressure or external fields [3]. The appearance of isosbestic points in the temperature-dependent absorption of pure solutes has been investigated by Morrey [2]. He discusses two mechanisms which influence the temperature dependence of electronic spectra. One is the variation in concentration due to volume contraction of the solvent which is a linear function of temperature [4]. The more interesting mechanism is the temperature dependence of the extinction coefficient. Although the conditions for the existence of isosbestic points have been given, the physical nature of the second mechanism has not been explained. We show that in favourable cases isosbestic points of this type correspond exactly to the energy of $J,m \leftarrow O,0$ transitions. $m$ and 0 denote vibrational levels of the electronic states J and O respectively. This means that in cases where isosbestic points are observed in the temperature-dependent electronic absorption spectra of pure molecules in solution, it is easy to locate the corresponding vibrational levels in the excited state. This is illustrated by the temperature-dependent spectra of the xanthene dyes pyronine-101-perchlorate and rhodamine-3B-perchlorate.

## 2. Experimental details

Pyronine-101-perchlorate (PY101) was synthesized following Reynolds [6] and purified by flash column chromatography on silica gel (Fluka, silica gel 60, 230–400

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mesh) with chloroform–methanol (1:1 and 3:1). Laser grade rhodamine-3B-perchlorate (RH3B) was purchased from Eastman-Kodak Co. and used without further purification. Solutions ($10^{-5}$ M) of PY101 in 95% ethanol (Uvasol, Merck, art. 973) and RH3B in $n$-butylchloride (Fluka, art. 19755) were prepared for the measurement of the absorption spectra. The spectra were recorded on a Philips PU8740 spectrometer, adapted for the use of an Oxford Instruments DN1704 liquid nitrogen cryostat. Special sample cells of fused quartz (Glasbläserei Möller, Zürich) were employed. Reference spectra of the solvents were taken at room temperature in the same sample cell.

## 3. Conditions for the existence of isosbestic points

The existence of isosbestic points results from the linear dependence of the absorption spectra [3]. Let us assume that a pure dye dissolves in an ideal solvent showing no change in refractive index with temperature and no change in volume. The change in shape with temperature can only be caused by the change in population of the vibrational levels of the electronic ground state. The electronic absorption spectrum $S(\bar{\lambda}, T)$ is thus represented by

$$S(\bar{\lambda}, T) = B_{01} N \sum_{i=0} \sum_{m=0} \frac{e^{-E_i/kT}}{Q_{vib}(T)} \langle i|m \rangle^2 \tag{1}$$

The index $i$ represents the vibrational levels in the electronic ground state and $m$ the vibrational levels in the electronically excited state. $Q_{vib}(T)$ is the vibrational partition function. The electronic transition moment $B_{01}$ and the number of molecules $N$ in the cross-section of the light path are constant provided that no conformational changes occur. The first term in eqn. (1) represents the vibronic population of the electronic ground state according to the Boltzmann distribution, and the second term describes the Franck–Condon transition probabilities to vibronic levels of the electronically excited state.

The condition for an isosbestic point at the position $\bar{\lambda}_a$

$$S(\bar{\lambda}_a, T_\alpha) = S(\bar{\lambda}_a, T_\beta) \tag{2}$$

must hold for any temperatures $T_\alpha$ and $T_\beta$. There is always a temperature $T_\alpha$ at which only the lowest vibrational level $n_0$ is populated, so that eqn. (3) holds

$$n_0(T_\alpha) = \frac{e^{-E_0/kT_\alpha}}{Q_{vib}(T_\alpha)} \approx N \tag{3}$$

Combining eqns. (1), (2) and (3) leads to the condition

$$\left[ \sum_{i=0} \sum_{m=0} n_i(T_\beta) \langle i|m \rangle^2 \right]_{\bar{\lambda}_a} \approx N \left[ \sum_{m=0} \langle 0|m \rangle^2 \right]_{\bar{\lambda}_a} \tag{4}$$

This is an interesting equation which is satisfied at any isosbestic point observed under the conditions assumed. The sum of all the molecules in the individual levels $n_i(T_\beta)$ is equal to the total number of dye molecules $N$

$$\sum_{i=0} n_i(T_\beta) = \sum_{i=0} \frac{e^{-E_i/kT_\beta}}{Q_{vib}(T_\beta)} = N \tag{5}$$

Therefore we can write

$$\left[ \sum_{i=0} n_i(T_\beta) \left\{ \sum_{m=0} (\langle i|m \rangle^2 - \langle 0|m \rangle^2) \right\} \right]_{\bar{\lambda}_a} \approx 0 \tag{6}$$

This equation holds for any temperature $T_\beta$. It can thus only be fulfilled in the trivial case $i = 0$ and much more interestingly for transitions $J, m \leftarrow O, i$ with $\langle i | m \rangle^2 = \langle 0 | m \rangle^2$. From this it follows that the position $\bar{\lambda}_a$ of an isosbestic point corresponds to the energy of a $J, m \leftarrow O, 0$ transition. As a consequence it can be used to determine the energy of the vibrational level $m$ in the excited state.

The spectra can be corrected in cases where volume and/or refractive index change in a continuous way with temperature. However, if phase transitions occur, the isosbestic points are expected to disappear and their disappearance can be used to characterize the phase transition.

## 4. Examples

A number of spectroscopic studies on xanthene dyes have been published recently for different reasons [7]. Emission-wavelength-dependent biexponential decay was observed in time-resolved fluorescence measurements carried out on rhodamine-3B, pyronine-B and rhodamine-101-ethylester in protic solvents of different viscosity but with similar dielectric properties [8]. Continuing these studies, we have measured the absorption spectra of these dyes in different solvents as a function of temperature and have observed isosbestic points under different conditions. As examples, we now describe some observations on PY101 (1) and RH3B (2). In both cases the absorption observed in the visible part of the spectrum can clearly be attributed to a single electronic transition. Mirror symmetry between absorption and emission is apparent and becomes more pronounced at lower temperatures for both dyes. A solvent which seems to behave almost ideally is $n$-butylchloride but ethanol can also be used. We therefore report data in these two solvents.
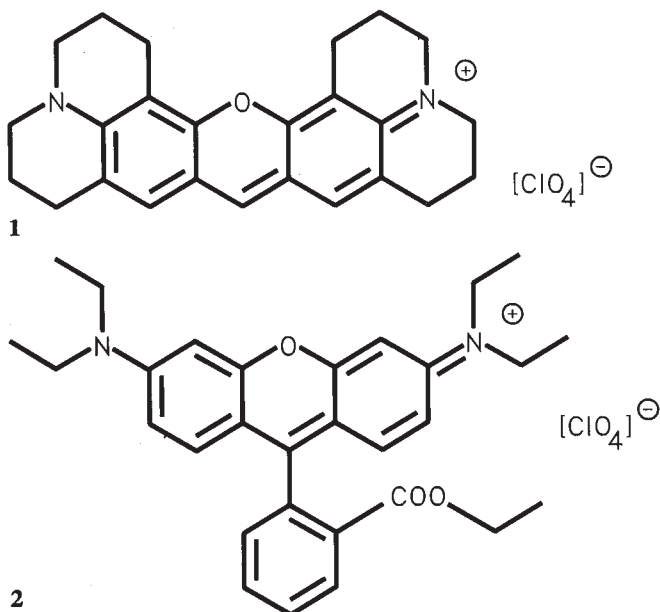


In Fig. 1 we show the absorption spectra of RH3B at different temperatures. The isosbestic points observed for RH3B and PY101 are reported in Table 1. The well-
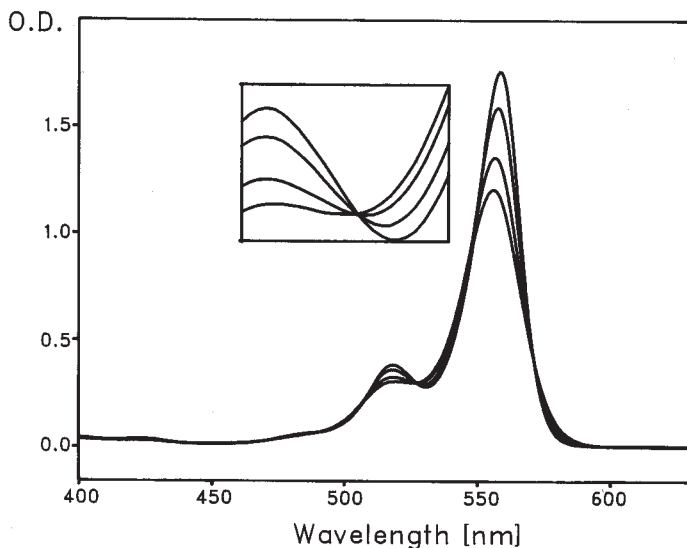
Fig. 1. Absorption spectra of rhodamine-3B-perchlorate ($10^{-5}$) in $n$-butylchloride at $-180$ (most intense absorption at maximum), $-140$, $-100$ and $-60$ °C. The isosbestic point at 529 nm is magnified.

TABLE 1

Isosbestic points of RH3B (20 to $-180$ °C) and PY101 (20 to $-100$ °C)

| Dye | $S_1 \leftarrow S_0$ $1,m \leftarrow 0,0$ $(\text{cm}^{-1})$ | Difference $m \leftarrow 0'$ $(\text{cm}^{-1})$ |
|---|---|---|
| RH3B | $0' \leftarrow 0,\ 17482$ | |
| | $m_1 \leftarrow 0,\ 18939$ | 1457 |
| | $m_2 \leftarrow 0,\ 19747$ | 2265 |
| | $m_3 \leftarrow 0,\ 20325$ | 2843 |
| | $m_4 \leftarrow 0,\ 21186$ | 3704 |
| PY101 | $0' \leftarrow 0,\ 16852$ | |
| | $m_1 \leftarrow 0,\ 18395$ | 1543 |
| | $m_2 \leftarrow 0,\ 18860$ | 2008 |
| | $m_3 \leftarrow 0,\ 19904$ | 3052 |

developed mirror symmetry between absorption and emission spectra [8] can be used to locate the $0' \leftarrow 0$ transition. The first isosbestic point corresponds to the $0' \leftarrow 0$ transition for both dyes; we expect that this should happen quite often, although we do not have the means to prove this statement. In the present case it enables the energies for the $m_i \leftarrow 0'$ vibrational transitions of the dyes in the $S_1$ state to be determined with good accuracy. This is illustrated in Fig. 2 and the vibrational frequencies are reported in Table 1. This remarkable result is expected to be useful in further studies of the excited state behaviour of these dyes.
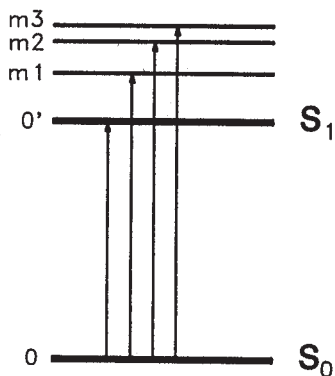
Fig. 2. $S_1 \leftarrow S_0$ transitions at low temperature and vibrational levels of the dyes in the $S_1$ state.

**Acknowledgments**

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