# Fluorescence and Phosphorescence Anisotropy Spectra of Indole in Poly(Vinyl Alcohol) Film at Room Temperature

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Excitation anisotropy spectra of indole were measured in poly(vinyl alcohol) film at room temperature. The behaviour of the phosphorescence anisotropy of indole in isotropic and anisotropic PVA film enabled a conclusion to be drawn that the $T_1 - S_0$ transition is located outside the indole ring plane, close to the perpendicular direction.

## 1. Introduction

Spectroscopic studies of indole are of interest when investigating the nature of the environment of tryptophan residues in model polypeptides and proteins [1]. As shown experimentally [2] and theoretically [3], the first and second excited states of indole are of $L_b$ and $L_a$ character, respectively (the Platt labels), i.e. the two neighbouring levels, $S_1$ and $S_2$, have different symmetries. The transfer of indole from non-polar to polar fluid media leads to a pronounced shift of the fluorescence spectra caused by the interaction with polar solvent molecules, with no corresponding shift in the absorption spectrum [4, 5]. Mataga et al. [4, 6] suggested that the $^1L_a$ state of indole lies energetically close to and above the $^1L_b$ state in absorption and possesses much more polar electronic structure than the $^1L_b$ state. The $^1L_a$ state is considerably better stabilized by the interaction with the polar solvent molecules than the $^1L_b$ state. Fluorescence of indole occurs both from $^1L_a$ and $^1L_b$ states [5–9], primarily from the $^1L_a$ state [10]. Valeur and Weber [10] have resolved the two absorption bands of the $^1L_a$ and $^1L_b$ states of indole based on fluorescence polarization experiments in propylene glycol at $-58\,^\circ C$. In this paper, fluorescence and phosphorescence excitation anisotropy spectra of indole in poly(vinyl alcohol) (PVA) film at room temperature are presented, and conclusions are drawn as to the transition moment directions in the molecule studied.

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## 2. Experimental

Isotropic PVA films were prepared at room temperature by introducing indole into aqueous PVA solution through methanol [11–13]. Anisotropic PVA films were obtained by stretching at about 350 K. Indole was purified by HPLC. The fluorescence spectrum and the steady-state anisotropy spectra of indole were measured as described by Lakowicz et al. [14]. Since the phosphorescence lifetime was of the order of 0.1 s, the phosphorescence spectrum was obtained using a simple phosphorescence. The excitation wavelength for the emission spectra measurements was 298 nm. The steady-state fluorescence and phosphorescence anisotropy spectra were measured for different excitation wavelengths, $\lambda_{exc}$, at a constant wavelength of observation, i.e. 320 nm and 435 nm, respectively.

## 3. Results and Discussion

The emission spectra of indole in an isotropic PVA film at 20 °C are shown in Figure 1. The ratio of the room temperature phosphorescence maximum at 435 nm to the fluorescence maximum at 320 nm is about 0.05. The background in the phosphorescence measurements was <1%. The fluorescence spectrum (290 ÷ 400 nm) is structureless, as in polar solvents [5],

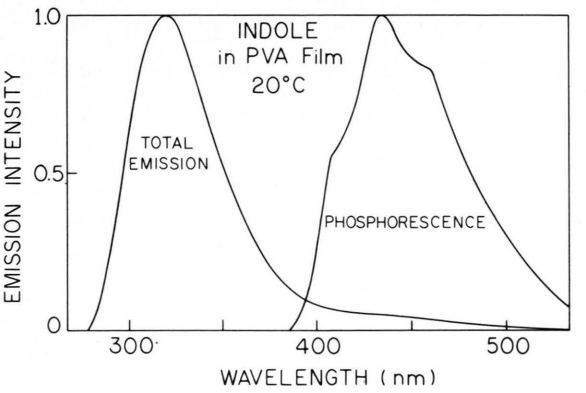


Fig. 1. Emission spectra of indole in PVA film at 20 °C.

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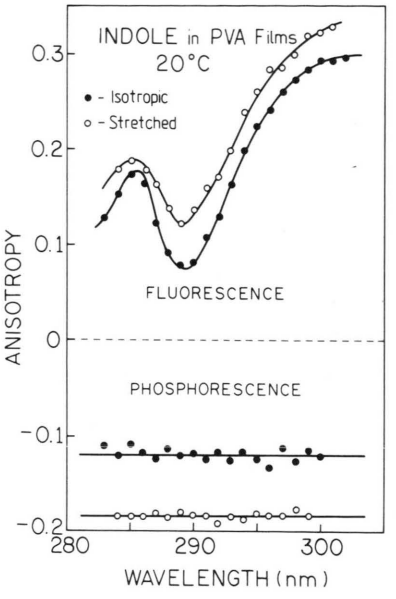


Fig. 2. Excitation anisotropy spectra of indole in isotropic and stretched PVA films at 20 °C.

while the phosphorescence spectrum (400 ÷ 520 nm) is slightly structured.

The fluorescence excitation anisotropy spectrum of indole in isotropic and stretched (stretch ratio $R_s \approx 12$; for the definition see [12]) PVA film (Fig. 2) is similar to that obtained in vitrified solutions [14]. The characteristic minimum is located at 289 nm. The phosphorescence anisotropy is negative and independent of the excitation wavelengths in the range of 284 ÷ 300 nm (Figure 2). In an isotropic PVA film the limiting emission anisotropy is only $r_0 = -0.12$. Such a value of $r_0$ results from limited rotational motions [15], which means that in PVA films treated as rigid media the indole molecules have little freedom for rotational motions. Such motions occurring in the PVA polymer can be effectively eliminated by stretching the film [16]. Upon the elimination of such restricted motions, the measured values of the limiting anisotropy of the molecule investigated are close to $r_0 = -0.2$ (Figure 2).

The $^1L_a$ and $^1L_b$ transition directions in indole are through to make an angle of 90° and are located in the plane of the molecule [10]. The result obtained of the phosphorescence anisotropy clearly shows that the $T_1 - S_0$ transition should be located out of the plane of the indole ring, close to the perpendicular direction.

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