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COMPARISON OF DIFFERENT MATHEMATICAL MODELS IN DECONVOLUTION OF LIGNIN FLUORESCENCE SPECTRA

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Abstract

We analysed fluorescence spectra of poplar lignin and lignin model compound, using gaussian, log-normal and exponential power model. Asymmetric models show wavelength positions of components in a complex molecule. Gaussian model indicates only discrete origin of the emission, but fails in defining component positions.

Introduction

Fluorescence spectroscopy is a sensitive tool for structural and kinetic studies of macromolecules. The analysis of fluorescent spectra of polymeric molecules is especially complex, since they may contain different fluorophores or a fluorophore in various microenvironments. Therefore, it is necessary to use computer methods for numerical deconvolution of complex emission spectra into individual components.

In this work we analyzed fluorescence spectra of lignin and lignin model compound, using three different mathematical models. The aim of the study was to see which of them best fits the original spectra, as well as to find out which model is most suitable for analytic application. Lignin, as a major structural polymer in the plant cell walls, is the second most abundant polymer on Earth. Fluorescence is an intrinsic property of lignin. The structural complexity of lignin makes its fluorescence spectra difficult to interpret. We also aimed to obtain the new data on the structural characteristics of lignin as a complex molecule and, on the basis of this approach, we hope to be able to distinguish lignins of different origin.

Materials and Methods

Lignin model dehydrogenative polymer (DHP) was synthesized from coniferyl alcohol, using horseradish peroxidase as an enzymatic catalyst [1].

Lignins from poplar (*Populus tremuloides*, clone 1214) and spruce (*Picea abies* (L.)) were obtained from wood mill using method of thyoacidolysis [2].

Fluorescence spectra were collected using a Fluorolog-3 spectrofluorimeter (Jobin Yvon Horiba, Paris, France) equipped with a 450 W xenon lamp and a photomultiplier tube. The spectra were corrected for the dark counts. In each measurement seven scans were averaged. The emission spectrum of the solvent (dioxane/water, 9/1 v/v) was subtracted. All measurements were performed at controlled temperature of 25 ºC by means of a Peltier element.

A total of $N_{DHP} = N_{poplar} = N_{spruce} = 22$ emission spectra were collected for each sample (DHP, lignin from poplar and spruce) by excitation at different wavelengths, starting from excitation maximum at 360 up to 465 nm, with a 5 nm-step. Nonlinear fitting of each of the 66 spectra was performed using the Nelder-Mead algorithm implemented in Matlab, version 6. All spectra were deconvoluted by gaussian, log-normal and exponential-power model:

$$I(\lambda) = \sum_{i=1}^{n} A_i \frac{1}{\sqrt{2\pi}\sigma_i} \exp \frac{-(\lambda - \lambda_{0i})^2}{2{\sigma_i}^2} \quad \text{Gaussian model}$$

$$\begin{cases} F_\lambda = F_m \exp\left\{-\left(\ln 2 / \ln^2 \rho\right) \ln^2 \left[\left(1/a' - 1/\lambda\right) / \left(1/a' - 1/\lambda_m\right)\right]\right\}, & for \lambda > a' \\ F_\lambda = 0, \qquad for \lambda \le a' \end{cases} \quad \text{Log-normal model}$$

$$F_\lambda = \frac{a}{\exp(b\lambda)} \frac{1}{\exp(c(\lambda^d)) - 1} \quad \text{Exponential-power model}$$

Results and Discussion

A set of fluorescence spectra, recorded by excitation of spruce lignin at different wavelengths (λ_{ex}), starting from excitation maximum at 360 nm, with 5 nm-step, is shown in Fig. 1 (left panel). A typical result of component Gaussian deconvolution of the emission spectra of spruce lignin, obtained by excitation at 405 nm, is shown in Figure 1 (right panel).

In order to acquire a distribution profile for all the positions of the components, we constructed corresponding histograms of their positions. Histogram profiles indicate that there are intervals of grouping for the position of the components. However, since positions and relative amplitudes of histogram maxima depended on the number of histogram abscissa intervals, we calculated approximate probability distribution (APD) for position components by weighed averaging histogram values for a set of histograms, where interval number varied from 2 to 30. Results of overlaid APDs for the poplar lignin, obtained using different mathematical models, are presented in Fig. 2. The presence of several peaks in APD in case of all lignin samples indicates that its fluorescence originates from several distinct fluorophores, which may be distinct molecular species or the same species in different microenvironments. We can note that there is a high compatibility between APD results obtained by the two asymmetric models.

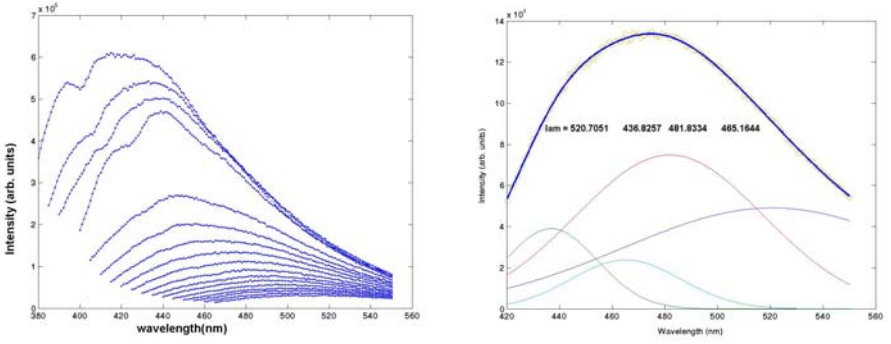

Fig. 1. Left panel: Emission spectra of poplar lignin dissolved in dioxane/water (9:1, v/v), for excitation wavelengths in the range 360 - 465 nm with 5 nm step. Right panel: Example of emission spectra deconvoluted into four Gaussian components; excitation wavelength was 405 nm; Circles – measured spectra, solid lines - separate components and their sum fitted to the experimental data.

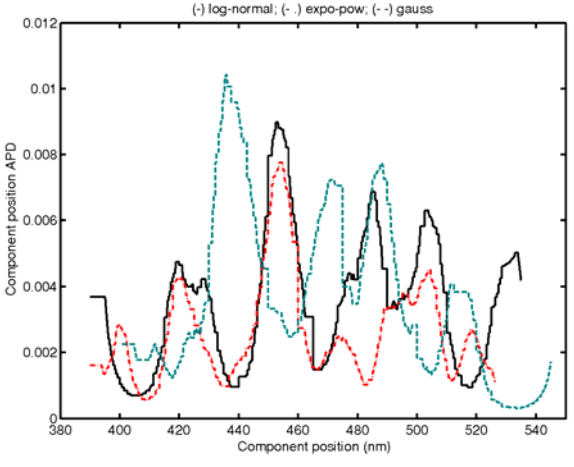

Fig. 2. Approximate distribution of the probability that a fitted Gaussian (---), exponential-power (-.-) and log-normal (—) component of all poplar emission spectra occupies a position on the λ_0-axis (reconstructed from the histograms). In all cases, APD is multimodal, indicating that component positions are not equally probable.

The obtained results show that asymmetric models, as more precise description of the emission spectrum of a fluorophore, show wavelength positions of components in a complex molecule. Gaussian model, however, indicates only discrete origin of the emission, but fails in defining component positions.

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