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Dimeric spectra analysis in Microsoft Excel: A comparative study

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ABSTRACT

The purpose of this work is to introduce the reader to an Add-in implementation, Decom. This implementation provides the whole processing requirements for analysis of dimeric spectra. General linear and nonlinear decomposition algorithms were integrated as an Excel Add-in for easy installation and usage. In this work, the results of several samples investigations were compared to those obtained by Datan.

1. Introduction

Self-association of dyes in solutions due to the attractive electrostatic forces is a well-known phenomenon [1–5]. The presence of dye aggregates plays an important role in solution chemistry and biological processes. The strength of the molecular association depends on several factors, such as the dye concentration and structure, temperature, solvent and other factors [6–8].

Due to the characteristic differences between the absorption spectra of the dye in the monomeric and aggregate forms, molecular association can be detected from the absorption spectra. Therefore, UV–Vis spectroscopy is one of the most applicable and successful techniques for investigating the aggregates [9,10].

The molecular aggregates have been the subject of renewed interest in recent years. Substantial studies on optical spectroscopy of aggregates show that the results obtained form various methods may differ significantly. Such a discrepancy may possibly involve several factors, such as use of different theoretical and technical procedures or employment of approximations in the analysis of experimental data [11].

Generally, analysis of dimer spectra consists of three steps: (1) defining mathematical model for dimerization, (2) fitting mathematical model to experimental data, and (3) describing the dimer structure and interactions between the two dye molecules. All mathematical models are similar for dimer formation (step 1), in addition all explanations about dimer structure and interactions apply similar theoretical basis defined by Kasha exciton model [12] (step 3). It means that the differences in the literature data might come from fitting procedure (step 2).

As the spectral profile contains large number of data, computerized fitting procedures have been preferentially utilized. Development of such procedures led to implementation of spectral analysis program into commercial packages [13]. Although, such powerful software packages are often suitable,

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they do not completely fit with all the requirements of different users. Additionally they often require preprocessing steps to each spectrum in a data collection that may initiate human inaccuracy.

The purpose of this paper is to introduce an Add-in implementation, into Microsoft Excel [14–20], as an alternative for processing requirements. Since the monomer and dimer species cannot be isolated, this Add-in can be used for estimating the species concentrations and spectra, as well as the equilibrium constant. In addition, comparison between obtained results through different methods confirms the reliability and efficiency of such an Add-in, i.e. Decom.

2. Computational methods and theory

2.1. Theory

All investigations on dimer formation employ the following molecular model:

$$2[M]\underset{K_{dissociation}}{\overset{K_{association}}{\rightleftarrows}}[D]$$

where [M] and [D] are monomer and dimer concentrations, respectively. Although, this equilibrium has been investigated in both forward and reverse directions, final decomposition results are identical, and equilibrium constants are inversely proportional. The dimer association constant (K_d) can be expressed as:

$$K_d = \frac{[D]}{[M]^2} \tag{1}$$

At the point, one should consider two more equations based on mass balance and total absorption:

$$\text{Total analytical concentration} \equiv [C] = [M] + 2[D] \tag{2}$$

moreover:

$$\begin{aligned} Abs_{\text{total}}(\lambda) &= Abs_{\text{monomer}}(\lambda) + Abs_{\text{dimer}}(\lambda) \\ &= \varepsilon_m(\lambda)\cdot[M]\cdot l + \varepsilon_d(\lambda)\cdot[D]\cdot l \end{aligned} \tag{3}$$

Here $\varepsilon_m(\lambda)$ and $\varepsilon_d(\lambda)$ correspond to molar absorptivities of monomer and dimer at a given wavelength, and "l" is the optical path length. Since this equilibrium exists even at very low concentration, pure molar absorptivities of monomer and dimer species at a particular wavelength and their concentrations cannot be directly determined. For N samples of known analytical concentrations [C], and k observed data points per spectrum (which involves k unknowns ε_m and k unknowns ε_d), we have kN experimental data and $2k+1$ unknowns (ε_m and ε_d at all k wavelengths, plus k_d). Three major methods including linear and nonlinear least squares fitting and decomposition of the absorption spectra matrix into a product of target and projection matrices [21] have been used for evaluation of these unknown parameters.

Several researchers [22,23] through different technical approach have used linear or nonlinear least squares fitting methods. These approaches mainly consist of two major parts including determination of the dimerization constant and spectra decomposition. The third approach previously has been described in details and implemented in Datan program that was used for comparison.

2.1.1. *Linear least squares fitting approach*

Dividing Eq. (3) by $[C]\times l$ and defining α as the fraction of dye molecules present as monomers:

$$\alpha = \frac{[M]}{[C]} \tag{4}$$

Combining Eq. (4) and Eq. (1) gives:

$$K_d = \frac{(1-\alpha)\cdot[C]}{2\cdot\alpha^2\cdot[C]^2} \tag{5}$$

$$\begin{aligned} \varepsilon_{\text{total}}(\lambda) &= \varepsilon_m(\lambda)\cdot\alpha + \varepsilon_d(\lambda)\cdot\left(\frac{1-\alpha}{2}\right) \\ &= \left(\varepsilon_m(\lambda) - \frac{\varepsilon_d(\lambda)}{2}\right)\cdot\alpha + \frac{\varepsilon_d(\lambda)}{2} \end{aligned} \tag{6}$$

Assuming that $\varepsilon_m(\lambda)$ and $\varepsilon_d(\lambda)$ are concentration independent, variation of $\varepsilon_{\text{total}}(\lambda)$ vs. α should yield a straight line with intercepts at $\varepsilon_m(\lambda)$ for $\alpha=1$ and $\varepsilon_d(\lambda)/2$ for $\alpha=0$. By systematic variation of K_d and analyzing the straight-line fitness in a series of wavelengths, one obtains optimum dimerization constant as well as monomer and dimer spectra.

2.1.2. *Nonlinear least squares fitting approach*

Introducing Eqs. (1) and (2) into Eq. (3) produces:

$$\begin{aligned} Abs_{\text{total}}(\lambda) &= \varepsilon_m(\lambda)\cdot\left(\frac{-1+(1+8\cdot K_d\cdot[C])^{1/2}}{8\cdot K_d}\right)\cdot l \\ &\quad + \varepsilon_d(\lambda)\cdot\left(\frac{[C]}{2} - \frac{-1+(1+8\cdot K_d\cdot[C])^{1/2}}{8\cdot K_d}\right)\cdot l \end{aligned} \tag{7}$$

Fitting the experimental data to Eq. (7), using nonlinear curve fitting method in a series of wavelengths, one obtains monomer and dimer spectra as well as optimum dimerization constant, simultaneously.

2.2. *Computational methods*

Most new spectrophotometers provide options for exporting spectral data directly into spreadsheets. Excel is a user-friendly spreadsheet medium with an internal programming language, which supports new application development [24–26]. We decided to implement specific linear and nonlinear modules for complete control on process. Such integration avoids some difficulties, which arise from direct processing in worksheet. In addition, it provides a way in which all input data, output results, and procedures could be handled by system memory.

Based on theoretical features, the flowchart illustrated in Fig. 1 was used for putting these methods into operation. As it can be seen, both fitting methods use the same approach for defining K_d and the main distinction is different linear and