Journal of Molecular Structure 1124 (2016) 221–227

Contents lists available at ScienceDirect

Journal of Molecular Structure

journal homepage: http://www.elsevier.com/locate/molstruc

Investigation on the spectral properties of 2D asynchronous fluorescence spectra generated by using variable excitation wavelengths as a perturbation

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A R T I C L E I N F O

Article history:
Received 19 October 2015
Received in revised form
18 January 2016
Accepted 18 January 2016
Available online 20 January 2016

Keywords:
Two-dimensional asynchronous
Spectroscopy
Micro-environment
Molecular aggregate
Anthracene
Fluorescence spectrum

A B S T R A C T

Properties of 2D asynchronous spectra generated from a series of fluorescence emission spectra are investigated. Variable excitation wavelengths are utilized as an external perturbation. Based on the results of mathematical analysis and computer simulation, we find that no cross peak will be produced on the 2D asynchronous spectrum if the fluorescent solute under investigation occurs in a single micro-environment. The observation of cross peaks implies that the fluorescent molecule may occur in different micro-environments in a solution. Based on these results, we use 2D asynchronous spectra to investigate the emission spectra of anthracene dissolved in cyclohexane. When the concentration of anthracene is low, no cross peak is produced in the resultant 2D asynchronous spectrum, confirming that anthracene is dissolved as single molecule in the solution. As the concentration elevated, cross peaks appear in the corresponding 2D asynchronous spectra. A plausible explanation of this phenomenon is that anthracene may undergo aggregation via π-π interaction or π-C-H interaction.

1. Introduction

Fluorescence spectroscopy has evolved rapidly during the past several decades. As a result, fluorescence spectroscopy is widely utilized as tool of investigation, analysis, control and diagnosis in many fields relevant to physical, chemical, biological and medical sciences [1,2]. Nowadays, fluorescence spectroscopy is a central technique for detecting trace amount of analytes, characterizing the dynamic structure of proteins and enzymes, and elucidating energy transfer in luminescent processes.

When fluorescent spectroscopic method is utilized to study a chemical system, a complex situation may be encountered. Even though only one fluorescent solute occurs in a solution, the fluorescent molecules may exist in different micro-environments, so that the corresponding emission spectra are different. That is to say, the variation in emission spectra may indicate that the fluorescent molecule occur in different micro-environment in a solution. This is very important in the study of molecular aggregate, excimer and energy transfer behavior of molecule in fluorescent process [2]. In an actual experiment, the obtained emission spectrum is the summation of spectra of the fluorescent molecule in different micro-environments. In many cases, variations on the emission spectra caused by fluorescent molecules existing in different micro-environments are quite subtle. Consequently, emission spectra of the fluorescent molecule in different micro-environments are highly overlapped, making it difficult to clearly judge whether the fluorescent molecules exist in different micro-environments or not.

Two-dimensional correlation spectroscopy, which is a powerful spectral analysis technique proposed by Noda in the 1980s [3,4], may provide a way to solve the problem. The technique is based on

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http://dx.doi.org/10.1016/j.molstruc.2016.01.047

the analysis of a set of spectral data, which are sequentially obtained under some form of perturbation applied on the sample. The spectra are then transformed into a correlation intensity plot on a spectral plane defined by two independent spectral variable axes. In 2D correlation spectroscopy, the spectral data are extended to the second dimension, providing a chance to alleviate the problem caused by overlapping spectral congestion [5–26].

In our previous work, we have applied two-dimensional spectroscopic methods to characterize intermolecular interaction between two solutes dissolved in the same solutions, leading to the so-called orthogonal sample design scheme (OSD) [27–32]. In the OSD approach, the portion of spectral signals that has nothing to do with intermolecular interaction is completely removed via the operation based on the multiplication of orthogonal vectors. As a result, intermolecular interaction that renders the spectral signal to deviate from the Beer-Lambert's Law can be manifested as cross peaks in 2D correlation spectra. Based on this original idea, we have developed asynchronous orthogonal sample design (AOSD) [33–35], double orthogonal sample design (DOSD) [36] and double asynchronous orthogonal sample design (DAOSD) [37–44], 2D asynchronous spectrum with auxiliary cross peaks (ASAP) [45] and other approaches [46,47] to reveal spectral variations on the characteristic peaks of solutes caused by intermolecular interactions. We find the idea of orthogonality is also helpful in the fluorescent spectroscopic studies.

In this paper, we investigate 2D asynchronous fluorescence spectrum generated by using variable wavelength of monochromatic radiation as an external perturbation. Mathematical analysis, computer simulation as well as experimental work on a real chemical system are conducted. We find that it is possible to combine the Kasha's rule of emission spectra and the mathematical property of the Hilbert-Noda matrix to simplify the resultant 2D asynchronous fluorescence spectrum. As a result, a new approach is proposed to judge whether the fluorescent molecule exists in a single environment or not.

2. Experimental

2.1. Reagent

Cyclohexane was of HPLC grade and purchased from Tianjin concord technology Co., Ltd. Anthracene with purity of 98% was purchased from Aladdin reagent Co., Ltd.

2.2. Apparatus

All the fluorescence spectra were recorded on a Hitachi F-7000 spectrofluorimeter. The emission slit was set as 1 nm in order to enhance the resolution of the emission spectra, the excitation slit was set as 5 nm and the scan speed was set as 30 nm/min to improve the signal-to-noise ratio of the emission spectra.

2.3. Model systems for mathematical analysis and computer simulation

Both absorption spectra and emission spectra of model systems are simulated. Two dimensional asynchronous spectra were constructed by using the simulated 1D spectra based on a program written in our lab using the software of MATLAB (The Math Works Inc.).

2.4. Real chemical system

Two dimensional asynchronous spectra were generated by using the aforementioned 1D spectra based on a program written in our lab using the software of MATLAB (The Math Works Inc.).

3. Results and discussion

The top-view of the fluorescent spectroscopic experiment is shown in Scheme 1. The sample solution is put in a cuvette whose length is *l*. The cuvette is inserted into a metal socket. Each corner of the cuvette is fixed by a metal holder in L-shape. The length of each side of the L-shaped holder is *a*. The sample solution in the red rectangle is directly excited by the incident light so that fluorescence can be produced. Because of the blockage of the L-shaped metal holder, fluorescence from the pink region and blue region cannot reach the detector of the spectrofluorimeter. In the following part, consequently, we only consider the fluorescence signal from the grey region of the sample solution in the cuvette, which eventually reaches the detector of the spectrofluorimeter.

Radiation from a Xenon lamp is applied on the sample solution in the cuvette and fluorescence is recorded on a direction perpendicular to the incident light. To simulate the light from the Xenon lamp, the intensity of the monochromatic radiation is modeled by using a Gaussian function as shown in Eq. (1).

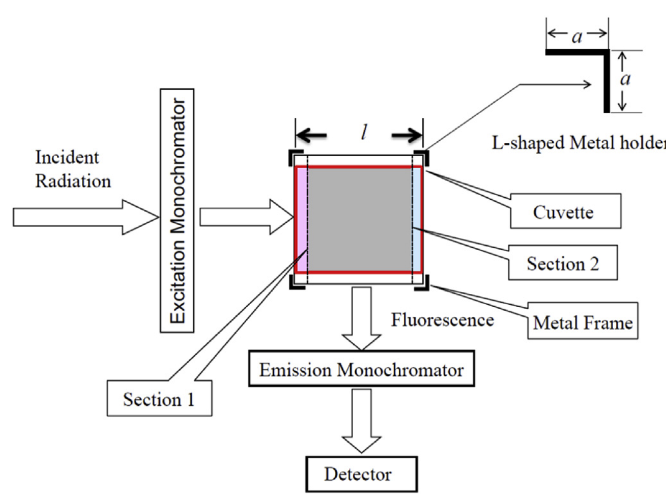
$$I(\lambda) = I_{max} e^{-(\ln 2)\left[(\lambda-\lambda_{max})^2/W^2\right]} \quad (1)$$

where λ is the wavelength of the radiation, I_{max} is the maximum light intensity, W is the half width at half-height (HWHH) of the emission band of light source, and the values of the above parameters are listed in Table 1.

Herein we discuss the spectral behavior of 2D asynchronous spectrum in two typical systems described in the following part.

3.1. The system where the Kasha's rule is valid

According to the literature [1], one basic property of emission spectra can be described by the well-known Kasha's rule. That is to say, the shape of emission spectra is invariant when the wavelength of the monochromatic excitation radiation is changed. We will discuss the spectral behavior of 2D asynchronous spectrum when the Kasha's rule is valid. Two-dimensional asynchronous spectrum was generated by using variable excitation wavelengths of the monochromatic radiation as an external perturbation. It should be pointed out that the Kasha's rule is valid when only one fluorescent



Scheme 1. The top view of the experimental setup for the measurement of fluorescence spectrum. It should be pointed out that only fluorescence from the grey region of the sample solution in the cuvette can reach the detector of the spectrofluorimeter.