

Determination Fluorescence Emission Spectra of Phenazine 5,10-Dioxide Using Multi-Peaks Gaussian Fitting Method in Different Solvent

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Abstract

Phenazine 5,10-dioxide has been synthesized by a reaction of phenazine with hydrogen peroxide (H₂O₂) in acetic acid (HAc) solution. And its fluorescence emission spectra has been determined in different solvent by overlap peaks of a principal peak with a wavelength (λ) at 520 - 545 nm and a shoulder peak with λ at 500 - 515 nm, which are respectively assigned to dimer and monomer emission peaks. The maximum wavelength, peak area, and other spectral parameters for phenazine 5,10-dioxide dimer and monomer were obtained from the analysis of overlap peaks by a multi-peaks Gaussian fitting method, then the value of relative integral emission intensity for dimer and monomer (I_2/I_1) in different solvents with various concentration can be obtained. When the concentration of phenazine 5,10-dioxide is low, the value of I_2/I_1 grows linearly with the concentration increase, and the maximum emission peaks of the monomer and dimer redly shift significantly. When the concentration of phenazine 5,10-dioxide is high, the value of I_2/I_1 grows with the concentration increase in tetrahydrofuran (THF), ethyl acetate, acetonitrile and ethanol, however it decreases with the concentration increase in benzene, toluene and acetic acid (HAc).

Keywords

Phenazine 5,10-Dioxide, Fluorescence Spectrum, Multi-Peaks Gaussian Fitting, Dimer, Overlap Peaks

吩嗪-5,10-二氮氧化物的合成及光谱性质的研究

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摘 要

在醋酸溶液中用过氧化氢氧化吩嗪合成吩嗪-5,10-二氮氧化物。测量了不同溶剂和浓度条件下吩嗪-5,10-二氮氧化物的荧光发射光谱。其特征是吩嗪-5,10-二氮氧化物二聚体(520~545 nm)发射峰以肩峰方式与单体(500~515 nm)主发射峰叠合在一起。通过高斯多峰拟合实现了单体与二聚体荧光发射光谱叠合峰的分峰拟合计算, 得到吩嗪-5,10-二氮氧化物单体与二聚体最大发射峰波长, 峰面积等光谱参数, 进而求得二聚体与单体相对积分发射强度(I_2/I_1)与浓度、溶剂的关系。当溶液浓度较低时二聚体/单体相对积分发射强度呈线性增大, 随着溶液浓度的增加, 单体与二聚体最大发射峰发生显著红移。在四氢呋喃、乙酸乙酯、乙腈、乙醇溶液中二聚体/单体的积分相对发射强度 I_2/I_1 随浓度的减少而减小, 但在苯、甲苯、冰乙酸溶液中随着溶液浓度的降低 I_2/I_1 反而增大。

关键词

吩嗪-5,10-二氮氧化物, 荧光光谱, 高斯多峰拟合, 单体, 二聚体

1. 引言

吩嗪-5,10-二氮氧化物及其衍生物具有很好的生物[1]-[3]、药理活性[4] [5], 对其光化学反应[6] [7]的研究引起人们的关注。吩嗪二氮氧化物对细胞有毒性并有抗癌作用[8]。Lavaggi 等[4] [9]将吩嗪-5,10-二氮氧化物衍生物作为低氧选择性疗法抗肿瘤前体药。Katsuyuki 等[3]认为是这类物质存在一种二氮氧化物的单电子还原态的活化形式, 它能够产生羟基自由基以及单线态氧离子。这种自由基对核酸链进行氧化破坏, 能够破坏低氧肿瘤细胞的 DNA, 从而达到治疗作用。吩嗪-5,10-二氮氧化物及其衍生物应用于药物化学[3] [4]及光化学[5]反应多见报道。但是, 有关吩嗪-5,10-二氮氧化物的荧光光谱的研究少有报道[6]。本文报道在醋酸溶液中用双氧水氧化吩嗪合成吩嗪-5,10-二氮氧化物, 对其进行了元素分析、¹HNMR 谱、红外光谱、荧光光谱表征。对其在不同浓度及溶剂中的荧光光谱并进行高斯多峰拟合分析[10]-[12], 讨论浓度及溶剂对单体与二聚体发射光谱的影响。仅用最大发射峰波长来表征化合物荧光发射光谱是有其缺陷的。当二聚体以肩峰形式出现在谱图中时, 将无法准确判断单体与二聚体最大发射峰具体的位置[13]-[16]; 而荧光发射光谱是高斯线型, 我们可以通过高斯多峰拟合将吩嗪-5,10-二氮氧化物单体与二聚体发射峰分开, 并求得其发射峰位及峰面积(积分发射强度)等光谱参数。由此研究浓度及不同溶剂对单体与二聚体发射光谱的影响, 从而研究吩嗪-5,10-二氮氧化物在不同溶剂中的聚集行为, 对揭示该类药物的药理特性具有重要意义。

2. 实验部分

2.1. 主要仪器与试剂

$^1\text{H-NMR}$ Bruker AVANCE AV 400 核磁共振波谱仪(瑞士 Bruker 公司); Tensor27 傅里叶变换红外光谱仪(德国 Bruker 公司)(KBr 做压片); EA2400 II 型元素分析仪(美国 PE 公司); F-4500 荧光分光光度计(日本日立公司); AL204-电子天平(梅特勒-托利多仪器(上海)有限公司); 吩嗪(Aldrich); 双氧水 30%、冰乙酸等试剂均为国产分析纯, 使用前均未进一步纯化。

2.2. 吩嗪-5,10-二氮氧化物的合成

吩嗪-5,10-二氮氧化物的合成路线[6] [17]如图 1。

在圆底烧瓶中加入吩嗪 0.3737 g, 双氧水 4 mL, 冰醋酸 30 mL, 搅拌下加热 60°C 回流 6 h 再加双氧水 4 mL 再回流 4 h, 冷却至室温抽滤得橙红色固体, 滤液减压除去溶剂。甲醇重结晶, 得橙红色固体。产率 78%。元素分析(%), $\text{C}_{12}\text{H}_8\text{N}_2\text{O}_2$ 实测值(计算值): C, 68.31(67.92); H, 3.62(3.80); N, 13.47(13.20)。 $^1\text{HNMR}$ (CDCl_3 , 400 MHz) 8.73(m, 4 H), 7.83(m, 4 H)。红外光谱(KBr, cm^{-1}): 3109 w, 1695 w, 1606 m, 1505 m, 1467 m, 1431 m, 1403 w, 1351 vs, 1270 m, 1209 w, 1115 w, 1091 s, 1010 w, 960 w, 856 m, 806 m, 764 vs, 619 s。

2.3. 荧光光谱的测量

设置狭缝宽度为 5 nm, 电压为 700 V, 激发波长为 470 nm, 扫描范围为 480~700 nm, 测量温度为 298.15 K。

2.4. 数据处理以及高斯多峰拟合

高斯多峰拟合操作如下: 启动 Origin, 导入荧光光谱, 绘制谱图。选择 Analysis/Fit Multi-Peaks/Gaussian, 设置峰个数为 2, 其他默认, 然后在单体和二聚体发射峰处分别双击, 系统自动完成高斯多峰拟合计算, 并输出 y_0 , I_1 , I_2 , w_1 , w_2 , x_{c1} , x_{c2} 等参数, 拟合结果如图 2。

其中 I_1 、 I_2 , w_1 、 w_2 , x_{c1} 、 x_{c2} 分别为吩嗪-5,10-二氮氧化物单体和二聚体的荧光发射强度、半峰宽和波长。

3. 结果与讨论

3.1. 浓度对荧光性质的影响

在溶液中, 吩嗪-5,10-二氮氧化物单体和二聚体共存, 如图 3。

在四氢呋喃溶液中当吩嗪-5,10-二氮氧化物浓度小于 $9.43 \times 10^{-5} \text{ mol/L}$ 时, 它的单体和二聚体荧光强度都随溶液浓度增大而增强如图 4(a)红线部分, 当溶液浓度大于 $9.43 \times 10^{-5} \text{ mol/L}$ 时它的单体和二聚体荧

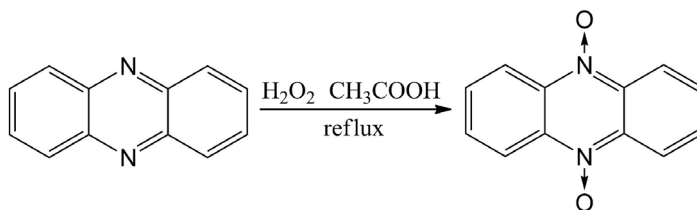


Figure 1. Synthesis of phenazine 5,10-dioxide
图 1. 吩嗪-5,10-二氮氧化物的合成路线

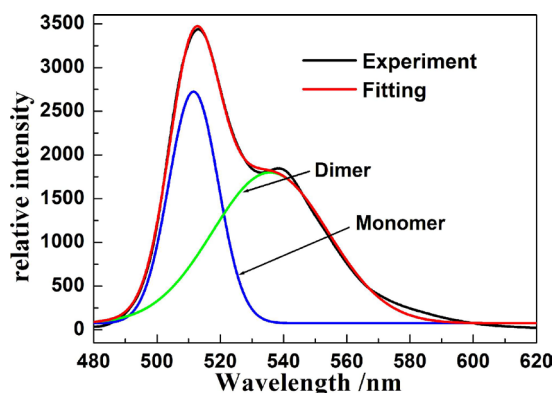


Figure 2. Multi-peaks Gaussian fitting on fluorescence spectra of phenazine 5,10-dioxide

图2. 高斯多峰拟合结果

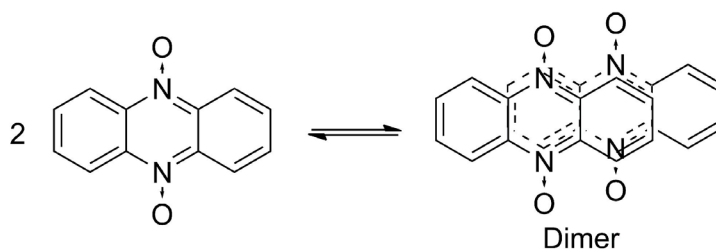


Figure 3. Chemical structure of phenazine 5,10-dioxide and its possible dimer [18]-[20]

图3. Phenazine 5,10-dioxide单体和可能的二聚体结构[18]-[20]

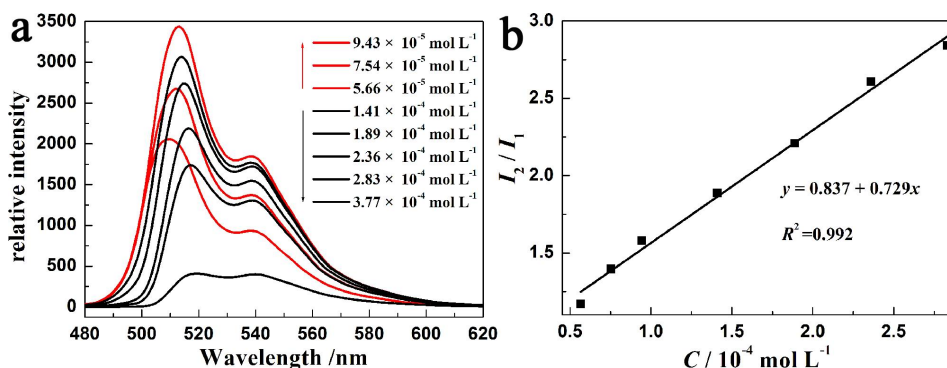


Figure 4. (a) Fluorescence spectra and (b) I_2/I_1 with phenazine 5,10-dioxide concentration in THF
图4. 四氢呋喃溶液中吩嗪-5,10-二氧化物浓度与(a)荧光强度和(b)二聚体/单体相对发射强度的关系

光强度随溶液浓度增大而减小如图 4(a)黑线部分, 这种浓度效应[21]-[23]可能由以下几种原因: 1) 内滤效应, 2) 入射光被液池前部的荧光物质吸收, 而使检测到的荧光强度降低。3) 浓度较高时产生荧光的激发态分子与基态分子的二聚物, 从而导致荧光强度的下降。当浓度更大时形成荧光物质的基态分子聚集体, 导致荧光强度更严重地下降。4) 浓度较高时增加分子之间的碰撞从而导致荧光猝灭。吩嗪-5,10-二氧化物浓度增大, 形成的二聚体增多, 溶液中的分子有序性增加, 单体与二聚体最大发射峰红移。

如图 4(b)所示, 二聚体积分发射强度/单体积分发射强度随着浓度的增大一直增大。当浓度小于 $3.77 \times 10^{-4} \text{ mol/L}$ 时 I_2/I_1 随浓度呈直线关系, 线性关系式为 $y = 0.837 + 0.729x$ 。二聚体随着吩嗪-5,10-二氧化物

物浓度的增大所占比例增大。

3.2. 溶剂的影响

图 5 是吩嗪-5,10-二氮氧化物在不同溶剂中的荧光发射光谱经高斯多峰拟合后的结果。溶剂对峰型的影响不大,对荧光强度有明显的影响,但影响规律不一。浓度大时在苯和甲苯中二聚体与单体发射峰强度最大。在图 6(a)中不同溶剂中吩嗪-5,10-二氮氧化物荧光发射光谱经分峰后单体与二聚体最大发射峰位置随浓度增大时波长红移,随着浓度的增大形成的二聚体增多,溶液中的分子有序性增加,单体与二聚体最大发射峰红移。在图 6(b)中四氢呋喃、乙酸乙酯、乙腈、乙醇溶液中 I_2/I_1 随浓度的降低而减小二聚体

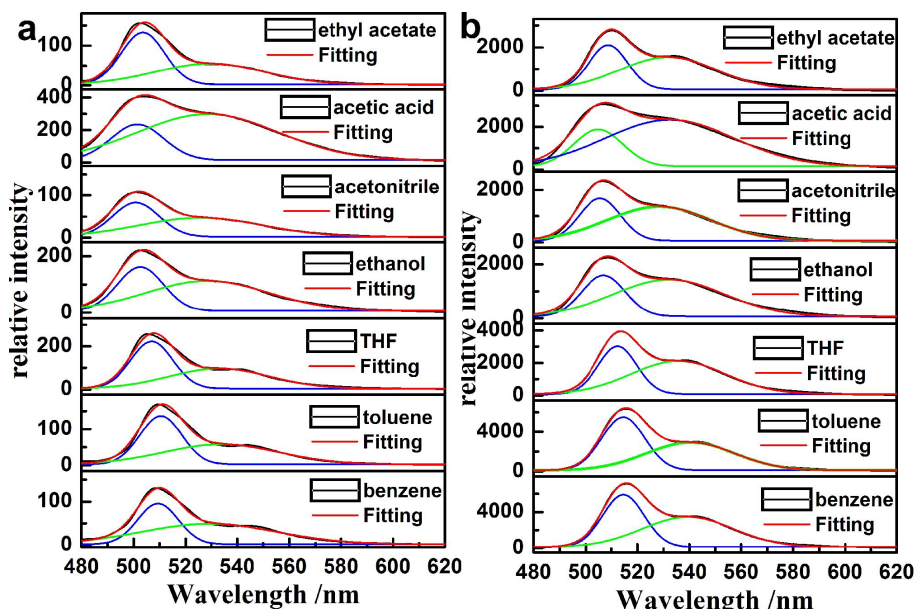


Figure 5. Fluorescence emission spectra of phenazine 5,10-dioxide in different solvents with different concentration of phenazine 5,10-dioxide (a) 3.77×10^{-6} mol/L and (b) 9.43×10^{-5} mol/L, λ_{ex} at 470 nm

图 5. 吩嗪-5,10-二氮氧化物在(a) 3.77×10^{-6} mol/L and (b) 9.43×10^{-5} mol/L 不同溶剂中的荧光光谱, $\lambda_{\text{ex}} = 470$ nm

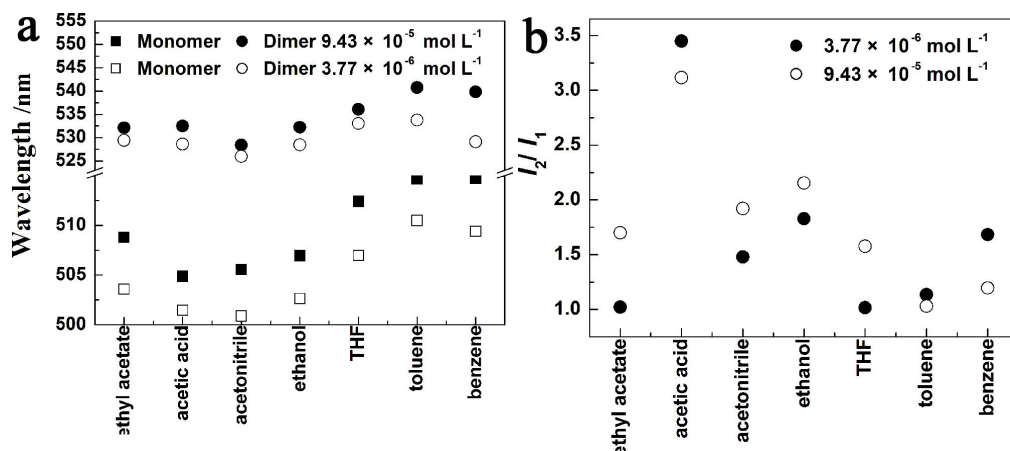


Figure 6. Variation of (a) peak fluorescence wavelength and (b) I_2/I_1 with concentration for phenazine 5,10-dioxide for different solvents

图 6. 溶剂对二聚体与单体最大发射峰的影响

所占的比例减少。有趣的是在苯、甲苯、冰乙酸溶液中随着溶液浓度的降低 I_2/I_1 反而增加, 可能的解释吩嗪-5,10-二氮氧化物是平面分子[9]根据相似相容原理吩嗪-5,10-二氮氧化物在苯与甲苯溶液里溶解性较好, 随着浓度减小, 单体碰撞的机会增加而出现碰撞猝灭, 单体积分发射光谱面积减小。二聚体体积大碰撞的机会少所以 I_2/I_1 反而增加。在乙酸溶液中可能是吩嗪-5,10-二氮氧化物与乙酸形成氢键, 二聚体荧光发射光谱效率增加峰面积增加所以随着溶液浓度的降低 I_2/I_1 反而增加。

4. 结论

在四氢呋喃溶剂中随着吩嗪-5,10-二氮氧化物浓度的增加, 单体和二聚体的荧光发射光谱波长红移, 强度都先增后减, 且单体与二聚体发射峰步调一致。二聚体对单体的积分荧光发射峰强度比随浓度的增大而增大, 当浓度小于 3.77×10^{-4} mol/L 时 I_2/I_1 随浓度呈线性关系, 线性关系式为 $y = 0.837 + 0.729x$ 。在不同溶剂中二聚体及单体的最大发射峰随浓度的增大而红移, 二聚体与单体的相对发射强度表现有趣的现象。在四氢呋喃、乙酸乙酯、乙腈、乙醇溶液中 I_2/I_1 随浓度的减少而减小, 但在苯、甲苯、冰乙酸溶液中随着溶液浓度的降低 I_2/I_1 反而增大。

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