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硕士 学位 论文

水溶性酞菁光学分子探针在分析科学  
的若干新应用研究

Several Original Applications of Water-soluble  
Phthalocyanines as Optical Probes in Analytical Sciences

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## 摘要

酞菁化合物大多性质稳定、易于合成、结构可修饰性强，可引入各类活性基团从而获得具有各种功能的衍生物。尤为难得的是，中心配位原子为反磁性的金属酞菁化合物大多具有荧光量子产率高、荧光发射在红色区域且可进行长波激发（因而可有效避开背景荧光和散射光的干扰）等优良荧光特性。本学位论文围绕金属酞菁作为光学分子探针在分析科学的应用而展开，共六章。

第一章 首先对酞菁的结构、基本性质与应用作了简要介绍。重点阐述酞菁在分析科学领域的应用，包括酞菁在催化动力学分析中的应用、酞菁作为光学探针的应用以及酞菁作为光学成像探针的应用等。

第二章 建立了铋离子的高特异性、高灵敏的定量分析新方法，并对反应机理进行了探讨。在酸性介质中，低浓度的多聚-L-赖氨酸（Poly-L-lysine, PLL）对四磺基铝酞菁具有高效荧光猝灭作用，形成几乎无荧光的离子对缔合物（AlS<sub>4</sub>Pc-PLL）。在Bi<sup>3+</sup>存在下，AlS<sub>4</sub>Pc-PLL的荧光显著恢复。进一步的金属离子筛选实验表明，只有Bi<sup>3+</sup>能够使AlS<sub>4</sub>Pc-PLL体系的荧光恢复，而其他金属离子的存在并不导致荧光恢复现象的发生，显示Bi<sup>3+</sup>对AlS<sub>4</sub>Pc-PLL的响应具有高度的特异性。据此，以AlS<sub>4</sub>Pc-PLL构成离子对红色荧光探针，建立了Bi<sup>3+</sup>的高特异性、高灵敏的定量分析新方法。方法用于实际样品的测定，获得了满意的结果。我们认为其反应机理类似于EDTA对铋离子的滴定反应。在较强酸性介质中，Bi<sup>3+</sup>与EDTA的螯合常数远大于其他金属离子，因而可在酸性介质中进行滴定（绝大多数其他金属离子不能在此介质中滴定）。PLL链上富含氮、氧配体，因而可以和Bi<sup>3+</sup>特异性结合成稳定的螯合物。

第三章 建立了氟离子含量的高灵敏高特异性快速测定新方法，并研究了磺基铝酞菁和氟离子之间的反应机理。在溶液中，氟离子和磺基铝酞菁的轴向配体竞争性结合中心配位金属原子铝，从而使原有的轴向配体被替代生成了磺基氟铝酞菁，溶液由蓝绿色变为蓝色，并在640 nm处出现一个新吸收峰。进一步的研究表明磺基铝酞菁对氟离子具有高选择性线性响应与裸眼识别功能。

第四章 建立了可测定纳克级RNA的高灵敏定量分析新方法。在弱碱性条件下，带有阴离子基团的RNA可与荷正电的四取代三甲基碘化铵铝酞菁

(Tetra(trimethylammionio) aluminum phthalocyanine, TTMAAlPc)发生静电作用而使TTMAAlPc沿RNA骨架发生聚集,导致TTMAAlPc的荧光显著猝灭,且猝灭程度与RNA含量呈线性正相关,因此可以用于RNA的检测。本法灵敏度高、线性范围宽、对RNA测定常见干扰物质包括阴、阳离子、表面活性剂和维生素等的抗干扰能力强,且操作简便。本研究还以参比法首次测定了不同pH下TTMAAlPc的荧光量子产率,结果显示TTMAAlPc的荧光量子产率不仅较高,而且对大范围的酸度保持稳定,表明TTMAAlPc是极具应用潜力的新型红色荧光探针,值得深入研究,开拓其应用。

**第五章 观察到TTMAAlPc的荧光行为受到DNA的显著影响,显示其与DNA存在相互作用。**采用吸收光谱、荧光光谱、荧光各向异性、电泳分析、无机离子竞争实验以及结合比、结合常数的计算,首次对阳离子铝酞菁TTMAAlPc与DNA相互作用的模式进行了较为系统的研究,结果表明,TTMAAlPc与DNA的相互作用模式是一种较强的静电结合模式。计算结果显示TTMAAlPc与DNA的结合比为 $n_{\text{TTMAAlPc}} : n_{\text{DNA}} = 1.2 : 1$ ,结合常数为 $1.95 \times 10^4 \text{ L mol}^{-1}$ 。研究结果为进一步探索阳离子酞菁类化合物与DNA的作用提供了理论参考,对该化合物在光化学诊治技术和荧光探针技术领域的应用具有积极意义。

**第六章 提出一种新的计算小分子-大分子相互作用结合常数的方法。**通过研究小分子荧光化合物与蛋白质、多糖、核酸的相互作用,提出了“荧光过猝灭”这一概念。讨论了这种现象发生的机制并提出作图法快速校正荧光猝灭滴定曲线。应用作图法对荧光滴定曲线进行校正,扣除“过猝灭”部分,再通过Stern-Volmer方程计算结合常数。将此方法应用于小分子与蛋白质、多糖、核酸结合常数的测定,以计算四磺基铝酞菁(Tetrasulfonated Aluminum Phthalocyanine, AlS<sub>4</sub>Pc)和人血清白蛋白(Human Serum Albumin, HSA)体系、吖啶橙(Acridine Orange, AO)和肝素钠(Heparin Sodium, Hep)体系、耐尔蓝A(Nile blue, NB)和小牛胸腺DNA(Calf Thymus DNA, ct DNA)三个体系的结合常数为例,证实了该方法的可靠性与准确性。

**关键词:** 铊菁; 铱离子; 氟离子; RNA; DNA; Stern-Volmer方程

## Abstract

Phthalocyanine compounds are stable compounds with facile way of synthesis. The parent structure of phthalocyanines can be modified by introducing active groups to obtain a variety of functional derivatives. Interestingly, when antimagnetic metal ions coordinated with phthalocyanine, the resulting metal phthalocyanines compounds turn to be fluorescent with high quantum yield. Fluorescent phthalocyanines which could be excited by longwavelength light, emit at red region. Therefore, scattering light and the background fluorescence could be effectively avoided. This work focus on the applications of water-soluble phthalocyanines used as optical probes in analytical sciences. It is divided into six chapters.

In chapter 1, the molecular structure, basic properties and applications of phthalocyanine compounds were reviewed. The main point of review focus on the applications of phthalocyanines in analytical sciences, including the following aspects: catalytical kinetic analysis, spectroscopic probes in quantitative analysis and optical probes for imaging.

In chapter 2, the main idea of this work is to develop a novel method for quantitative determination of bismuth ion with high specificity and sensitivity. We have observed that in acid media, a low concentration of poly-L-lysine (PLL) has a strong fluorescence-quenching effect on AlS<sub>4</sub>Pc, forming the ion-pair complex as AlS<sub>4</sub>Pc-PLL with almost no fluorescence. However, in the presence of Bi<sup>3+</sup>, the fluorescence of AlS<sub>4</sub>Pc-PLL dramatically recovers and the emission is visual because of the remarkable recovery. Screening experiments with other metal ions reveal that only Bi<sup>3+</sup> can restore the fluorescence of the AlS<sub>4</sub>Pc-PLL complex. The presence of other metal ions does not result in the recovery of fluorescence, indicating the high specificity of the response to Bi<sup>3+</sup> of AlS<sub>4</sub>Pc-PLL. This is the key finding of this study. It was also observed that the response to Bi<sup>3+</sup> of AlS<sub>4</sub>Pc-PLL exhibits a linear relationship over a large concentration range (three orders of magnitude). Based on these findings, we have established a new

quantitative analytical method for Bi<sup>3+</sup> with high specificity and high sensitivity, using the ion-pair AlS<sub>4</sub>Pc-PLL complex as a red-fluorescent probe, and we discuss the reaction mechanism. The method established addresses the complex operation and time-consuming problems in traditional methods and is thus suitable for real applications. Satisfactory results have been obtained when the method was applied to the measurement of real samples. This study further expands the scope of new applications of phthalocyanine-based red-fluorescent probes in analytical sciences.

In chapter 3, a new method for highly specific and sensitive determination of fluorine ion was proposed employing sulphonated aluminum phthalocyanine as an optical probe. The mechanism was also investigated. In aqueous phase, F<sup>-</sup> competes with the original axial ligand of AlS<sub>n</sub>Pc, chlorine atom, for the central coordination atom, aluminium. As a result, the original axial ligand atom was replaced and new product, F-AlS<sub>n</sub>Pc, was produced. The color of the solution turned from blue to blue-green, and a new absorption peak at 640nm appeared. Further research indicates that the constructed method can realise visual observation for F<sup>-</sup> due to its excellent specificity and high sensitivity.

In chapter 4, we develop a novel spectrophotometric method for highly selective determination of RNA on nanogram level. We have found that the fluorescence of TTMAAlPc could be dramatically quenched by RNA in weak alkaline media, and the degree of quenching has a linear relationship with RNA in a wide concentration range. This method has been applied to the analysis of practical samples with satisfied results. The constructed method is of high sensitivity and has a wide linear range, it also shows strong ability in the tolerance of foreign substances from anions, cations, surfactants and vitamins, all of which are common interferences encountered in the determination of RNA. Besides, it is the first report of the fluorescence quantum yield of TTMAAlPc which has been measured at different pH by reference method in this work. The achieved data indicates that the fluorescence quantum yield of TTMAAlPc is large and it keeps constant in a wide range of acidity, implying that TTMAAlPc is a high-quality red-emitting fluorescence probe, it has great potential for practical applications.

In chapter 5, we had observed that the fluorescence behavior of TTMAAlPc was significantly influenced in the presence of DNA. The interaction between this cationic phthalocyanine and DNA was systematically studied employing absorption spectra, fluorescence spectra, fluorescence anisotropy, electrophoresis, inorganic ion competition experiments, the calculation of binding ratio and binding constants. The results showed that the interaction between TTMAAlPc and DNA is an electrostatic model, but this effect is different from the conventional electrostatic interaction mode, it is a strong electrostatic binding mode. The calculation showed that the combination ratio of TTMAAlPc and DNA is  $n_{\text{TTMAAlPc}}: n_{\text{DNA}} = 1.2: 1$ , the binding constant is  $1.95 \cdot 10^4 \text{ mol}^{-1}$ . This research provides a theoretical reference for the further exploration of interaction between the cationic phthalocyanines and nucleic acids.

In the final chapter, we develop a novel graphic method for rapid correction of fluorescence quenching titration curves. We propose the notion of "fluorescence over-quenching" based on the interactions between small-molecule fluorescent compounds and nucleic acids, proteins, or biological polysaccharides. We discuss the mechanisms of this phenomenon and suggest a new method for the rapid correction of the fluorescence quenching titration curve via graphical methods. After the correction, the Stern-Volmer equation was used to calculate the binding constant. The system of interactions between small molecules and proteins, polysaccharides, or nucleic acids (i.e., tetrasulfonated aluminum phthalocyanine ( $\text{AlS}_4\text{Pc}$ )-human serum albumin (HSA), acridine orange (AO)-heparin sodium (Hep), or Nile blue A (NB)-calf thymus DNA (ct DNA) system) was investigated. The binding constants were calculated according to this novel method with good results. This study is an important tool for determining binding constants. It provides a theoretical and experimental basis for the determination of binding constants in fluorescence over-quenching systems and is of great value for the calculation of binding constants in small molecule-biomacromolecule interactions.

**Keywords:** phthalocyanine; bismuth ion; fluorine ion; RNA; DNA; Stern-Volmer equation

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