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A novel fluorescence ratiometric pH sensor based on covalently immobilized piperazinyl-1,8-napthalimide and benzothioxanthene

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Abstract

Two compounds with a terminal double bond, *N*-allyl-4-piperazinyl-1,8-naphthalimide (APN) and *N*-(2-methacryloxyethyl)benzo [*k*,*l*]thioxanthene-3,4-dicarboximide (MBTD), a benzothioxanthene derivative, have been synthesized as fluorophores for hydrogen ions sensing. To avoid the leakage of the fluorophores, the APN (a pH-sensitive carrier) and MBTD (a pH-insensitive carrier) which served as a referencing fluorophore were co-polymerized with acrylamide, hydroxyethyl methacrylate and triethylene glycol dimethacrylate on the glass surface treated with a silanizing agent. The ratiometric method can offset the influence caused by the external environment and instrument conditions and can improve the measuring precision. The fluorescence enhancement of APN with an increase in hydrogen ions concentration is based on the hindering of photo-induced electron transfer from aliphatic amine group to the napthalimide after its protonation. The proposed pH sensor shows sufficient selectivity, repeatability and short response time. The sensing membrane shows a good stability with a lifetime of at least 1 month. The sensor can be used for the determination of pH between 5.80 and 8.80. Most commonly co-existing inorganic ions and some organic species do not interfere with the determination of pH. The sensor has been applied to the analysis of water and soil samples.

Keywords: pH; Ratiometric fluorescence sensor; Naphthalimide; Benzothioxanthene; Covalently immobilization

1. Introduction

Recent years have seen the growing interest in the research and development of fluorescence pH sensors for acidity monitoring especially in biological fluids and cells, medicine analyses, environmental monitoring and other fields, in which the fluorescence pH chemical sensors play a significant role for the pH value can provide important information of the object characters. Many fluorescence pH sensors have been reported in the literatures. These sensors are based on the measurement of fluorescence intensity [1–9], fluorescence intensity ratio at two emission wavelengths [10–12] or fluorescence lifetime [13–15]. The most widely used techniques are the measurements of fluorescence intensity. Unfortunately, its accuracy is often compromised by instrumentation condition and external interferences, such as fluctuations in source intensity, the leakage of fluorophores, background fluorescence. Lifetime-based measurements are superior because lifetime is not affected by intensity. However, the complexity and demands on instrumentation restricted their wide application. The measurement of the ratio of fluorescence intensities at two different wavelengths, namely the ratiometric detection, provides an alternative approach to circumvent the problems associated with intensity-based measurements.

Naphthalimides are excellent fluorophores with high stability and quantum yield. Naphthalimides have been used as laser dyes [16,17], intracellular biomarkers [18], DNA photocleavers [19], electro-luminescent co-polymers and model compounds for photo-induced electron transfer [20–22]. A

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systematic study of naphthalimides used as fluorescent carriers has been undertaken in our laboratory [23–25]. Gan et al. [26] synthesized *N*-methyl-4-piperazinyl-1,8-naphthalimide and studied the photo-induced electron transfer process of the compound. The 1,8-naphthalimide structure is a fluorescing one that can emit fluorescence. If a strong electron-donating group, such as aliphatic amino group, is connected by a carbon chain, the fluorescence of the molecule would be quenched for the photo-induced electron transfer (PET) process. When the aliphatic amino group is protonated, the process of PET is forbidden and the fluorescence of 1,8-naphthalimide is restored. *N*-Allyl-4-piperazinyl-1,8-napthalimide (APN) has been synthesized by the present authors from 4-bromo-1,8-napthalic anhydride via imination reaction with allylamine, nucleophilic substitution reaction with piperazine. It has been observed experimentally that the APN shows faint fluorescence in base solution but strong fluorescence in acid solution. It has been proved that the APN had favorable response and excellent selectivity to the pH and can be used as a fluorescent carrier for a fluorescence pH sensor.

Benzothioxanthene is a kind of fluorescent dye with strong fluorescence and high light stability as well as good heat stability. It has been used as a plastic dye, a fluorescent paint and a photoconductor layer [27–29]. The authors synthesized *N*-(2-hydroxyethyl)benzo[*k*,*l*]thioxanthene-3,4-dicarboximide (HBTD) by referring to literature [30] with some modifications through imidation, Ullmann condensation and Pschorr ring close reaction with 4-brome-1,8-naphthalic anhydride as a starting material. For introducing into the molecule, a carbon chain with a terminal double bond capable to co-polymerize with monomers on the modified sensor surface, HBTD was reacted with methacrylic chloride and *N*-(2-methacryloxyethyl)benzo[*k*,*l*]thioxanthene-3,4-dicarboximide (MBTD) was synthesized. It is insensitive to the variation of pH and can be used as a reference fluorophore to compensate for the effects caused by external environment and instrument conditions.

In this paper, the authors developed a novel method for the fabrication of ratiometric fluorescence pH sensor. APN (a pH-sensitive carrier) and MBTD (a pH-insensitive carrier, used as a referencing fluorophore) were co-polymerized under UV radiation with acrylamide, hydroxyethyl methacrylate and triethylene glycol dimethacrylate on the glass surface treated with a silanizing agent.

The fluorescence enhancement of APN by protonation can be utilized for pH monitoring of solutions. When the sensing membrane contacted with different pH solutions, the fluorescent intensities of the sensing membrane were measured at excitation and emission wavelengths of APN and MBTD, respectively. The pH of solutions was related to the ratio of their fluorescence intensities.

In this way, a novel fluorescence ratiometric pH sensor was developed based on the covalently immobilized fluorophores. It possesses high sensitivity and selectivity, excellent stabilization, good reversibility and rapid response. All of these prove that the ratiometric method is feasible.

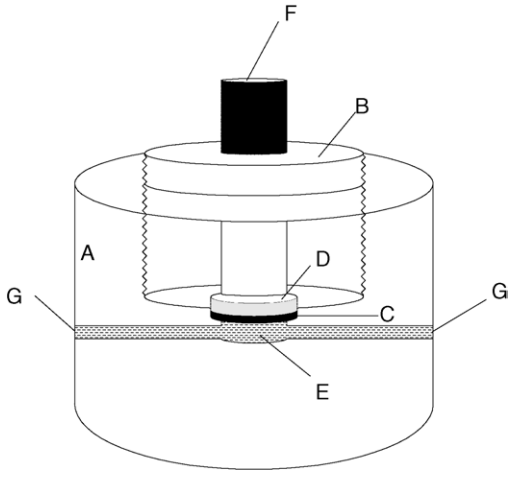

Fig. 1. Schematic diagram of the flow-cell: (A) flow-cell body, (B) mounting screw nut, (C) sensing membrane covalently immobilized on glass plate, (D) glass plate, (E) detecting chamber, (F) bifurcated optical fiber and (G) inlet and outlet channel for sample solution.

2. Experimental

2.1. Apparatus

All fluorescence measurements were conducted on a Perkin-Elmer LS-55 spectrofluorimeter with both excitation and emission slits set at 5 nm. The light source is a pulsed Xe lamp. A home-made poly(tetrafluoroethylene) flow-cell (Fig. 1) and a bifurcated fluorescence fiber were used for the pH sensing. The excitation light was carried to the cell through one arm of the bifurcated optical fiber and the emission light was collected through the other. A glass plate (diameter 13 mm) covered with a sensing membrane was fixed on the top of the flow chamber by the mounting screw nut with the membrane in contact with the sample supplied by a peristaltic pump (Shanghai Zhixin Instruments, Shanghai) at a flow rate of 3.0 ml min^{-1}. A PHS-3B pH meter (Shanghai Analytical Instruments, Shanghai) was used for pH measurements.

2.2. Materials

4-Bromo-1,8-naphthalic anhydride, purchased from Taizhou Chemicals (Zhejiang, China), was recrystallized twice from chlorobenzene (mp 218–220 °C). 3-(Trimethoxysilyl)propyl methacrylate (TSPM) was purchased from ACROS (Sweden). Piperazin was obtained as a gift from Xinxiang Jujing Chemical Ltd. (Henan, China, the purification of 99%). 2-Aminobenzenthiol was given as a gift by Shouerfu Chemical Co. Ltd. (Zhejiang, China) and used as the received (purity 99.22%). Allylamine was synthesized from allyl alcohol, hydrobromic acid and sodium thiocyanate by a three-step reaction [31]. Methacrylic chloride was synthesized according to reference [32]