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# Fluorescent mesoporous organosilicas for selective monitoring of $Hg^{2+}$ and $Fe^{3+}$ ions in water and living cells

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ABSTRACT

A new fluorescent mesoporous organosilicas bearing ethidium bromide in the framework was conveniently synthesized using 3-aminopropyltriethoxysilane as bridging organosilane functionality. Powder X-ray diffraction, $N_2$ sorption, transmission electron microscopy and scanning electron microscopy, and UV–visible absorption and fluorescence spectroscopy have been employed to characterize the materials. This material showed a selective monitoring of $Hg^{2+}$ and $Fe^{3+}$ ions with fluorescence quenching and visible color transition over a wide range of tested metal ions, which is due to the selective interactions of $Hg^{2+}$ and $Fe^{3+}$ ions with integrated functional groups at its pore wall framework. The experimental measurements reveal that the mesoporous organosilica material shows $Hg^{2+}$ and $Fe^{3+}$ ion selectivity upon addition of various concentrations of other competitive metal ions. Thus, the synthesized mesoporous organosilicas could monitor $Hg^{2+}$ and $Fe^{3+}$ ions in water and biological cells even in the trace amount.

## 1. Introduction

Fluorescent materials can recognize guest-species selectively by real-time monitoring or imaging [1]. The design of high performance novel fluorescent silica materials for the screening of heavy metal and toxic metal ions in environmental and biological species has attracted considerable research attention. The routine contamination of the environment and aquatic ecosystems with heavy and toxic metal ions has deleterious effects on the health of humans and animals [2]. The accumulation of toxic metal ions, such as $Hg^{2+}$, $Pb^{2+}$, $Cd^{2+}$ and $Cr^{3+}$, in the vital organs and tissues causes serious damage to the body organs, central nervous system and endocrine system [3]. On the other hand, $Cu^{2+}$, $Zn^{2+}$ and $Fe^{3+}$ are essential trace elements involved in the metabolic process of living beings, ranging from bacteria to mammals [4], but they can also have toxic effects when their concentrations exceed a certain limit [5]. Drinking water is a common source of heavy metal intake for humans. Heavy metal pollution in drinking water is caused mainly from the contamination of waste streams from industry, and is a serious threat to human health and ecological systems. Therefore, there is a need to develop material to monitor toxic metal ions in water or living cells and tissues. Mesoporous organosilicas are a robust tool for the sensing and quantification of metal ions of environmental and biological interest compared to conventional analytical methods, such as flame or plasma techniques, which can have low precision and sensitivity due to the effects of high back-ground and chemical interference [6]. Fluorescent [7] and colorimetric probes [8] have attracted considerable attention because they allow rapid detection using a simple apparatus and easy monitoring of metal ions in solution by the naked eye and in living cells by fluorescence microscopy. On the other hand, the majority of existing fluorescent/colorimetric probes are based mainly on organic molecules that make them quite inconvenient for potential practical applications considering cost-effectiveness because they are difficult to recover and reuse due to their homogeneity. The emerging heterogeneous fluorescent materials, such as quantum dots [9], fluorescent probe-modified silica nanowires [10] and mesoporous silica (MCM-41, SBA-15) nanoparticles [11], are applicable because of their stability, good optical transparency in the visible region, favorable biocompatibility, and recyclability, which make them a promising candidate for practical applications. Several studies have reported that mesoporous silica nanomaterials can be endocytosed efficiently by mammalian cells [12]. On the other hand, most of these materials required several tedious modification steps and appropriate fluorophores for their further utility. Until now, few mesoporous silica-based fluorescent materials have been reported for the monitoring of metal ions [13]. Of

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these, the majority can detect only a single metal ion by fluorescent quenching or fluorescent enhancement mechanisms [14].

Mesoporous organosilica materials (MOS) are a new class of functional materials with organic–inorganic hybrid frameworks that are synthesized from bridged organosilane functional precursors $[(R'O)_3\text{–Si–R–Si–}(OR')_3]$ [15]. Using this approach, a broad spectrum of functional moieties can be incorporated into the stable framework for the desired applications. In such a way, fluorescent chromophores can be incorporated into the mesopore walls rather than mesopore surfaces, which is an attractive approach to increasing the functional content loading with a high surface area and well ordered mesostructures. The fixing of fluorophores within the silica framework could yield a novel class of fluorescent mesoporous organosilicas with impressive advantages, such as fluorophore stability, to greatly reduce the level of fluorophore aggregation. Therefore, the fluorescent behavior of the resulting material is enhanced efficiently [16]. The development of a material that allows the simultaneous detection of multi-ions from bio-systems or eco-systems using a single entity has attracted considerable interest for current and future applications. Until now, there are no reports of the detection of multiple-ions, such as toxic cations and biological cations (e.g. $Hg^{2+}$ and $Fe^{3+}$), using a fluorescent mesoporous organosilica device.

This paper reports the synthesis of a new fluorescent mesoporous organosilica system for the screening of $Hg^{2+}$, $Fe^{3+}$ by a single entity through considerable fluorescent quenching and an optical color transition that can be distinguished by the naked eye. To achieve this, ethidium bromide (EB), was chosen as a fluorescent tag to synthesize a new ethidium bromide-bridged fluorescent mesoporous organosilicas. The synthesized mesoporous organosilicas might show combined dual colorimetric and fluorescence quenching properties upon interacting with specific metal ions. Metal ions/molecules can diffuse efficiently through the mesochannels of the hybrid materials owing to their large surface area and low-steric hindrance, resulting in the rapid accessibility and response of analytes [17,18]. Screening experiments of the fluorescent EB-MOS revealed the selective interaction of $Hg^{2+}$ and $Fe^{3+}$ ions through fluorescent quenching and distinguishable optical color transition mechanisms. The heterogeneous fluorescent mesoporous organosilica sensor reported in this paper has the following advantages over the existing homogeneous sensor probes, such as simple synthesis, fast and selective sensing response, membrane permeability, non-toxic nature, and recyclability. Although a number of organic fluorescent probes have been reported, the majority have shortcomings for practical applications, such as low water solubility, narrow pH range, and cytotoxicity of the ligand. The heterogeneous mesoporous organosilica sensor has the potential to circumvent the key technological gap as a new strategy for examining environmental and intracellular signaling biology.

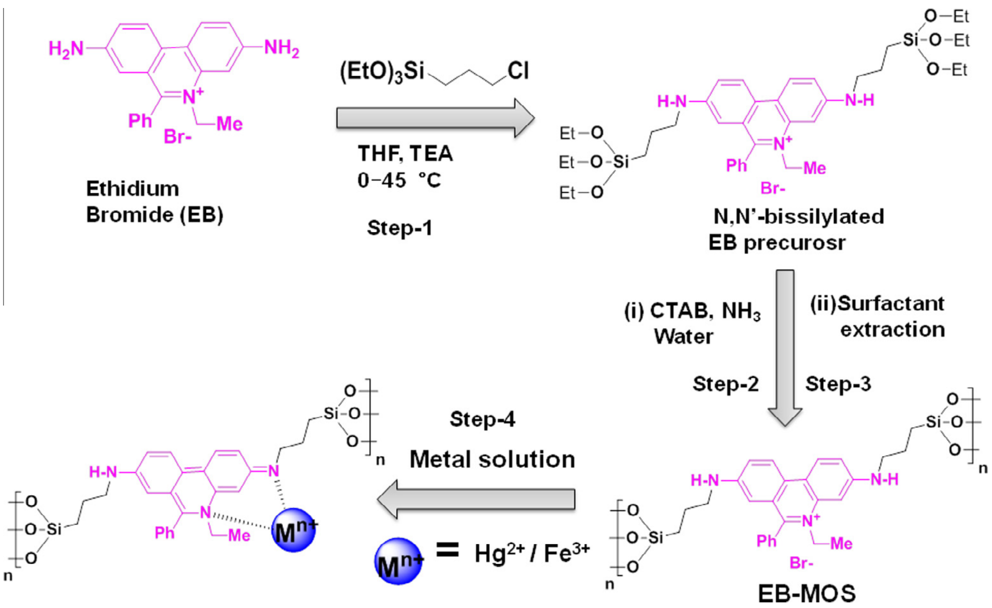
## 2. Experimental

### 2.1. Materials and reagents

Tetraethyl orthosilicate (TEOS), cetyltrimethylammonium bromide (CTAB), 3-chloropropyltriethoxysilane, ammonia solution (28%), ethydium bromide (EB) and triethylamine were purchased from Aldrich, USA. All the metal sources used in this study were obtained from Aldrich, USA. All the chemicals and reagents were used as received without any further purifications.

### 2.2. Synthesis of ethidium bromide-bridged alkoxysilane precursor (EB-precursor)

The ethidium bromide-bridged alkoxysilane precursor was synthesized, as shown in Scheme 1. A solution of ethidium bromide (0.5 g, 1.26 mmol) was prepared in 60 ml of dry tetrahydrofuran (THF). To this solution, 3-chloropropyltriethoxysilane (0.65 g, 2.66 mmol) was added drop wise with vigorous stirring under a nitrogen atmosphere at 0 °C. Subsequently, the reaction mixture was stirred for a further 12 h in the presence of a catalytic amount of triethylamine. The reaction progress was monitored by thin layer chromatography (TLC). After reaction completion, the reaction mixture was concentrated under reduced pressure. The unreacted components were removed by washing with diethyl ether and dried overnight in a vacuum to produce a dark pink oil (yield: 78%) (Scheme 1). $^1$H NMR (400 MHz, MeOH-$d_4$): $\delta$ 0.56 (t, 4H, $SiCH_2$), $\delta$ 1.14 (t, 18H, $CH_2CH_2CHO$), $\delta$ 1.47 (t, 4H, $SiCH_2CH_2$), $\delta$ 3.25 (t, 2H, NH), $\delta$ 4.32 (t, 12H, $CH_3$), $\delta$ 7.8–8.5 (m, 18H, aromatic). $^{13}$C NMR



**Scheme 1.** Synthetic routes of the bissilylated EB precursor (Step-1), EB-MOS chemosensor (Step-2) and a schematic diagram for the interaction of metal ions with EB functionalities (Step-4).