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Methylmercury Determination

The Determination of Methylmercury in Real Samples Using Organically Capped Mesoporous Inorganic Materials Capable of Signal Amplification**

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Mercury exists in the environment in a variety of compounds, and the toxicity depends on the chemical species.[1,2] Organomercury derivatives, especially methylmercury (CH_3Hg^+), are more toxic than inorganic or elemental mercury. Methylmercury is rarely emitted anthropogenically, but usually formed naturally through biomethylation of mercury, often of anthropogenic origin. Methylmercury subsequently bio-accumulates through the food chain, for example in the tissue of fish,[3] in which methylmercury concentrations are frequently found that exceed the maximum levels recommended by the Environmental Protection Agency (EPA) and the World Health Organization (WHO) for human consumption (0.1 and 0.23 μg (kg body weight)$^{-1}$ d^{-1}).[4] Methylmercury exposure in adults has been linked to cardiovascular diseases, autoimmune effects, hearing impairment, blindness, and death.[1] In a number of cases, mercury intoxication is related to the consumption of fish.[5]

Several analytical methods have been described for the determination of methylmercury in biological samples. For example, gas chromatography (GC) with electron capture detection (ECD)[6] or inductively coupled plasma mass spectrometry (ICP-MS)[7] and high performance liquid chromatography (HPLC) with elemental[8] or ICP-MS[9] detection have been extensively used. As an alternative to these technically sophisticated methods, which require a laboratory setting, the development of more simple procedures for in situ and rapid screening applications that are based on optical,[10] electrochemical,[11] or gravimetric[12] procedures have recently received considerable attention; these methods involve in part biological species as active sensing elements.[13]

Regarding the development of chromo- and fluorogenic indication systems for mercury derivatives, a large number of examples have been reported for the inorganic form (Hg^{2+}),[14–17] but few studies have targeted CH_3Hg^+.[18] Furthermore, most of these studies were unable to discriminate between Hg^{2+} and CH_3Hg^+ and did not involve the determination of the analyte(s) in relevant samples or matrices such as fish.[19]

Chemically, the great majority of the reported approaches rely on indicator molecules that either bind[14,17a] or react[15,17b] with Hg^{2+} to yield the desired change in color or fluorescence. Only very recently, alternative procedures involving organic, inorganic, or hybrid materials have been proposed, which are promising in their performance.[16] Our interest in the latter type of materials motivated us to explore bioinspired strategies toward new signaling models. For mercury indication, we combined our experience in Hg^{2+} sensing[17] and supramolecular hybrid materials design[20] and developed an organically capped mesoporous inorganic material for selective CH_3Hg^+ determination through signal amplification.

Inspired by gated ion channels and pumps, the proposed sensing mechanism relies on the opening of a pore that is controlled by the interaction of a certain molecular stimulus (the target species, CH_3Hg^+) at the receptors that close the gate.[21] Although this reaction itself can already induce an optical response, a second process is implemented in the system that leads to strong signal amplification: the pores of the hybrid are loaded with a large amount of dye molecules, which are only liberated upon analyte-induced opening of the pores. To date, apart from a few examples of analyte-induced pore blockage,[22] pore-opening methods for sensing applications have not been reported.[23]

The sensing procedure is shown in Scheme 1. The inorganic support is a calcined MCM-41 mesoporous solid that features homogeneous porosity, facile surface functionalization, inertness, and a high loading capacity.[24] The solid is first loaded with a dye (safranine O) and is then capped with 2,4-bis(4-dialkylaminophenyl)-3-hydroxy-4-alkylsulfanylcyclobut-2-enone (APC) groups. The APC moieties are

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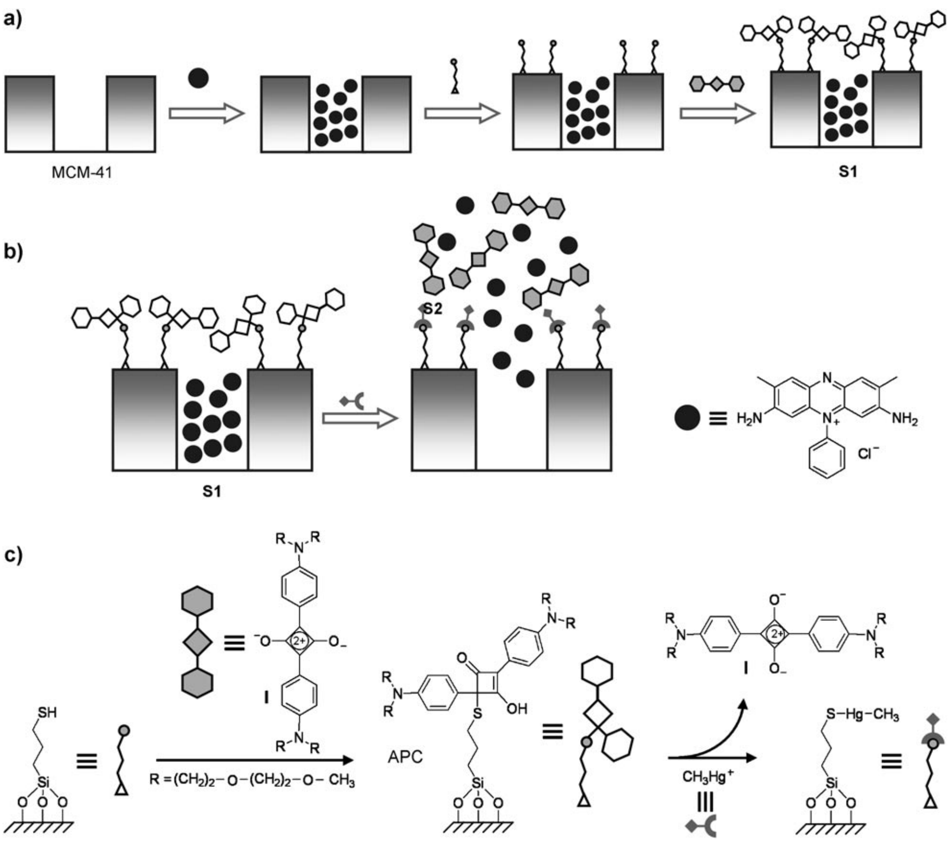

Scheme 1. a) Synthesis of material **S1**. b) Chromogenic detection of methylmercury ions using solid **S1** with safranine molecules (black circles) entrapped by APC groups (gray). c) Molecular basis of the capping (APC formation) and methylmercury-promoted uncapping steps (release of squaraine).

obtained in situ by a nucleophilic addition reaction between mercaptopropyl groups previously attached to the silica surface and the squaraine derivative **I**.[25] Once reacted, the bulky APC groups significantly inhibit dye release by closing the pores.[26] As we have previously shown, APC moieties can react with mercury, resulting in the coordination of the cation to the thiol group and liberation of the restored squaraine chromophore.[17b,27] This concept suggests that the system should work equally well with thiophilic CH_3Hg^+ as a "key" to unlock the "gate" (Scheme 1).

The mesoporous support was prepared following known procedures.[31] The MCM-41 structure of the starting material was confirmed by X-ray diffraction (Figure 1) and TEM (see the Supporting Information). The N_2 adsorption–desorption isotherms showed a typical type IV curve, with a specific surface area of 1249 $m^2 g^{-1}$, a narrow pore-size distribution, and an average pore diameter of 2.5 nm. The inorganic scaffold was loaded with safranine O as the reporter dye, and the surface of the mesoporous support was then functionalized with (3-mercaptopropyl)trimethoxysilane (MPTS). This sequence ensures that MPTS diffusion into the pores is hampered and that functionalization occurs primarily at the outer surface. The capped material **S1** was then obtained by reaction of the thiol groups with **I** in 5:1 v/v acetonitrile/CHES buffer pH 9.6 solution (see the Supporting Information). Figure 1 shows the powder X-ray pattern of **S1** with the expected features of the MCM-41 phase, indicating that after the filling of the pores and the functionalization of the surface, the mesoporous structure still remains unaltered. The mesoporous structure of **S1** is also clearly observed in the TEM image (Figure 2). From thermogravimetric and elemental analysis, a content of 0.032 $mmol\,g^{-1}$ SiO_2 of thio-derivatives and 0.47 $mmol\,g^{-1}$ SiO_2 of safranine were found.

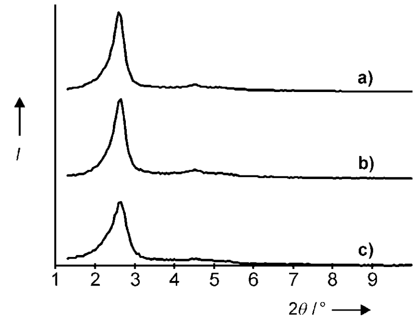

Figure 1. Powder X-ray patterns of a) MCM-41 as synthesized, b) calcined MCM-41, and c) **S1** hybrid material.

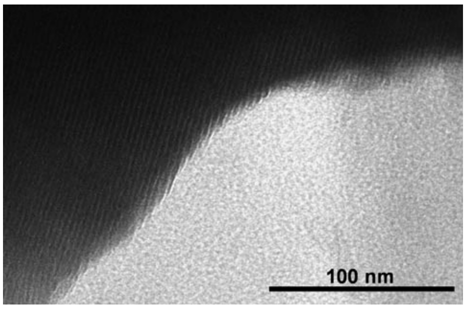

Figure 2. TEM image of solid **S1**, showing typical features of the hexagonal MCM-41 matrix.

Studies on the methylmercury-induced uncapping were carried out in acetonitrile/toluene 4:1 v/v. This solvent mixture was chosen because it fulfils two purposes: it achieves rather tight pore closure,[26] and this mixture is suitable for adopting the AOAC-approved discrimination between CH_3Hg^+ and Hg^{2+} by lipophilicity partitioning and thus the design of a protocol for the determination of CH_3Hg^+ in real samples. Furthermore, CH_3Hg^+ can be readily obtained from fish tissue by extraction in toluene, and acetonitrile promotes the solubility of the safranine dye upon release. In a typical preliminary experiment, **S1** was suspended in acetonitrile/toluene 4:1 v/v in the presence of 1.5 ppm CH_3Hg^+ and the suspension was stirred for 5 min (until maximum delivery of the safranine dye was observed). The delivery was monitored through the absorption band of safranine at 523 nm. Figure 3 shows that in the absence of CH_3Hg^+, some release of the entrapped dye is observed. However, the safranine band is significantly enhanced in the presence of 1.5 ppm of CH_3Hg^+, which is ascribed to the uncapping process upon reaction of CH_3Hg^+ with APC moieties.

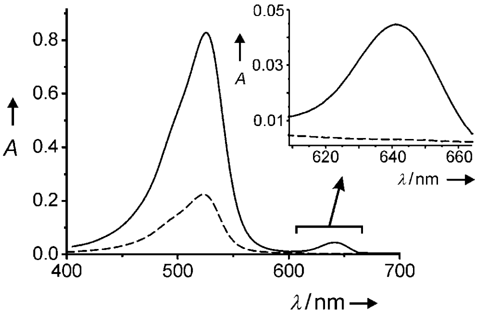

Figure 3. The absorption band of safranine (523 nm) in mixtures of acetonitrile/toluene 4:1 v/v in the presence of 1.5 ppm of CH_3Hg^+ (——) and in the absence of CH_3Hg^+ (– – –). Inset: The region of squaraine absorption in the presence (——) and absence (– – –) of CH_3Hg^+.

One of the key advantages of gated and loaded porous materials is their inherent signal amplification. Only very few analyte molecules, such as CH_3Hg^+, reacting at the pore openings are necessary to release a large number of signaling units (safranine) that were stored in the ensemble. In the present case, signal amplification is directly evident from a comparison of the ratios of the safranine band at 523 nm and the squaraine band at 641 nm (Figure 3). Taking into account the molar absorption coefficients of both dyes the removal of one APC group, that is, formation of one squaraine molecule, results in the delivery of about 200 safranine molecules.

Figure 4 shows typical spectrophoto- and fluorometric titration curves for safranine release. The increase in intensity of both the absorption and emission of safranine is proportional to the concentration of CH_3Hg^+, which is in agreement with the uncapping described above. The chromogenic indication reaction allows the detection of CH_3Hg^+ down to 0.5 ppm, and the use of standard fluorometric methods reduces the detection limit to less than 2 ppb.

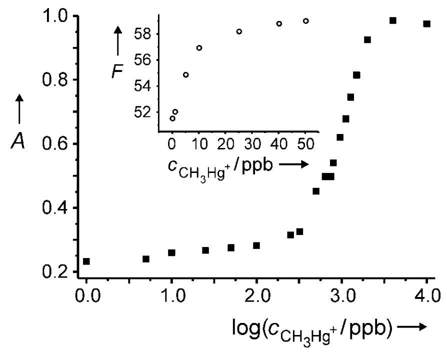

Figure 4. Spectrophotometric titration curves for safranine release monitored at 523 nm in acetonitrile/toluene 4:1 v/v in the presence of increasing amounts of CH_3Hg^+. Inset: Corresponding titration curve obtained from the emission intensities at 610 nm ($\lambda_{exc} = 523$ nm).

Based on this promising proof-of-principle, we extended our studies to real samples and attempted to achieve discrimination of CH_3Hg^+ from Hg^{2+} by lipophilicity partitioning. Approved analytical protocols rely on the selective extraction of CH_3Hg^+ from fish samples after treatment of the tissue with acid and toluene. To test the sensing ability of **S1**, a certified material of tuna fish ERM-CE464 with a known content of 5.50 ± 0.1 mg kg^{-1} CH_3Hg^+ was used. The extraction of methylmercury from the sample was carried out following the standard method proposed by the AOAC, namely, a mild acidic digestion of the sample followed by toluene extraction (see the Supporting Information).[28] The final toluene extract (5 mL) was then mixed with 20 mL of acetonitrile containing 10 mg of **S1**. The suspension was removed with a Teflon filter, and the fluorescence of safranine upon excitation at 523 nm was recorded. Following this procedure, the concentration of CH_3Hg^+ in the certified material was determined to be 5.69 ± 0.50 mg kg^{-1} of CH_3Hg^+ ($N = 3$ measurements) using a standard addition method, that corroborated well with the certified content. As a control experiment, the same procedure was carried out with samples of ERM-CE464 additionally spiked with 9.0 mg kg^{-1} of Hg^{2+}. Remarkably, a very similar response of **S1** was found in this case when compared with neat ERM-CE464 samples that lack inorganic mercury; that is, **S1** can selectively indicate 5.5 mg kg^{-1} CH_3Hg^+ and discriminate against Hg^{2+}. To assess the potential interference by other species, ERM-CE464 samples were also spiked with 40 mg kg^{-1} of the cations Na^+, K^+, Ca^{2+}, Mg^{2+},Cu^{2+}, Ni^{2+}, Zn^{2+}, Ag^+, Pb^{2+}, Cd^{2+}, Au^{3+}, and Tl^+ (as chloride, nitrate, or perchlorate salts) and with various organic species (sodium lauryl sulphate, cysteine, and histamine at 40 mg kg^{-1} and ethanol, heptylamine, and hexanethiol at 8 mg kg^{-1}); none of these species affected the response of **S1** toward CH_3Hg^+.

In conclusion, we have prepared a new capped mesoporous hybrid material that allows the selective chromo- and fluorogenic determination of methylmercury. The material was designed bearing in mind the affinity of thiophilic CH_3Hg^+ toward the capping APC groups. Signal amplification was implemented into the system by use of safranine O. The use of lipophilicity partitioning guaranteed the required selectivity for CH_3Hg^+, which has not yet been accomplished for other low-cost and time-effective sensory methods, whilst reaching comparable sensitivity in the ppb range.[11–13] This sensitivity is related to the signal amplification features of this method. Although laboratory-based ultra-trace techniques reach lower detection limits in the ppt range,[29] the method proposed herein competes well with current standard techniques[6–9] and with integrated instruments[30] with respect to detection limits and recovery rates for CH_3Hg^+ determination in fish matrices. The versatility and simplicity of the approach becomes obvious when considering that **S1** can be embedded into a hydrophobic sensing matrix, which can, for example, be dipped into the suspended and digested fish sample, thus extracting and indicating the lipophilic target species. Finally, the system can easily be adopted for ratiometric detection schemes, for example by assessing the fluorescence of safranine upon excitation with 488 or 532 nm and by monitoring the squaraine emission upon 633 or 638 nm excitation, that is, with common laser or LED light sources. We believe that these modular functionalized gated hybrid materials hold great promise for the development of new chromo- and fluorogenic sensing protocols.

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