Anion Sensors

Host Solids Containing Nanoscale Anion-Binding Pockets and Their Use in Selective Sensing Displacement Assays**

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The development of chromogenic and fluorogenic chemosensors for molecular sensing is an area of current interest.[1,2] The most widely used protocol in this field has been the

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binding site/signaling subunit approach, in which molecular coordination sites are covalently attached to chromogenic/fluorogenic reporters which transduce guest coordination into optical signals. Recently, two additional methodologies have been developed. One is the chemodosimeter approach, which is related to the use of guest-induced irreversible reactions,[2] and the other is the displacement protocol, which also involves the use of a binding site and a signaling reporter, although here the two subunits are not covalently attached but form a molecular ensemble.[3–5] In the latter systems addition of a certain guest to solutions of the sensing ensemble results in a displacement reaction: the binding site coordinates to the guest, and the signaling subunit is released. This method, inspired by displacement reactions in immunoassay protocols,[6] has been recently reported for the sensing of small anions with very good results.[7,8] This approach has several advantages. For instance, the noncovalent anchoring of binding sites and indicator groups allows a large number of combinations to be tested with minimum effort in order to obtain tuned sensing systems. Furthermore, these sensing ensembles usually operate in pure water or water/organic solvent mixtures. However, the displacement approach also has several limitations. One restriction is that the spectroscopic characteristics of the indicator in the molecular ensemble must be different to those in its noncoordinated form.[3] Another disadvantage is that sometimes it requires extensive and time-consuming synthetic efforts to finally obtain somewhat complex receptors.

All of these systems are based on the use of molecular anion hosts[2] having in common the presence of binding sites placed in suitable cavities that fit in size and charge with the guest to be coordinated and sensed. These binding pockets have typically been obtained by the use of 1,3,5-trisubstituted 2,4,6-triethylbenzene scaffolds[7i,8a,8e–8h] or cages incorporating metal complexes.[7b–h,8c,8d,8i] Following a different approach, we and others recently reported that adequately functionalized nanosized cavities in mesoporous solids can somehow mimic active-site cavities found in biological systems.[9–13] Our aim was to demonstrate that functionalized pores in mesoporous solids could act as binding pockets and be used as anion hosts in chromogenic displacement assays. The protocol is schematically shown in Figure 1. Suitable nanosized pores are functionalized with appropriate binding sites. The functionalized solid is then loaded with a dye capable of coordinative interaction with the anchored coordination sites. In the presence of a target anion displacement of the dye into the solution is achieved and results in colorimetric detection of the guest.

To check this idea, host solids **S2** and **S3** containing nanoscale cavities with anion-binding sites were prepared and loaded with suitable dyes. The mesoporous starting material UVM-7[14] was first treated with an excess of 3-aminopropyltriethoxysilane to yield solid **S1** (Scheme 1). Solid **S2** was obtained by reaction of **S1** with an excess of 2-methylthio-2-imidazoline hydroiodide in refluxing methanol for 16 h.[15] Solid **S3** was synthesized by reaction of **S1** with mannose in the presence of sodium cyanoborohydride.[16] Both solids were exhaustively washed and dried (40% yield). Solid **S2** was then converted to solid **S2-1** by loading with the dye

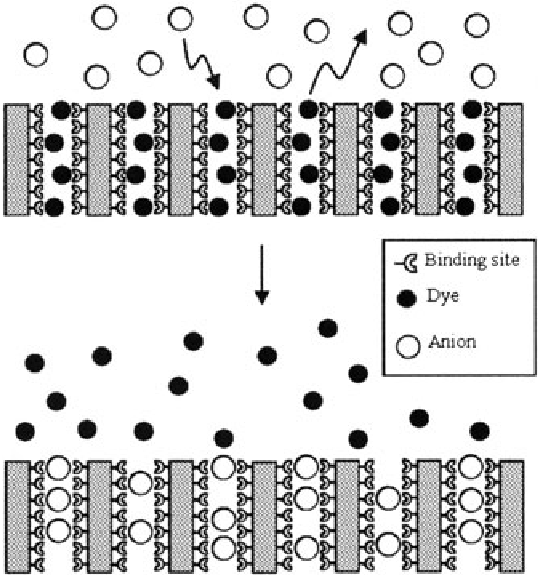
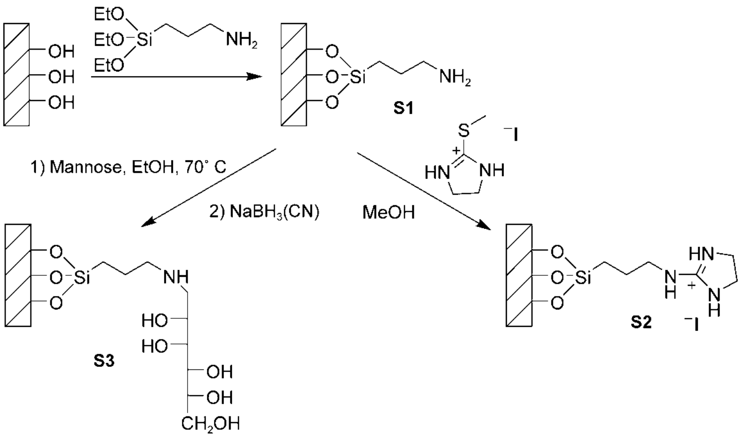
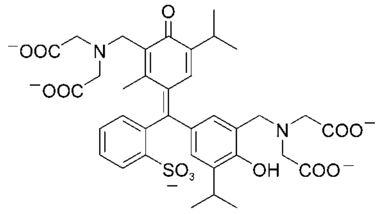


Figure 1. Idealized representation of the use of functionalized mesoporous materials in colorimetric displacement assays.



Scheme 1. Synthesis of solids **S1**, **S2**, and **S3**.

Methylthymol blue (**1**), which contains four carboxy groups and is expected to form strong complexes with guanidinium groups.[17] In a similar manner, solid **S3** was loaded with azoic dye **2** to give solid **S3-2**.[18] This dye was selected because of



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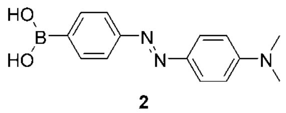

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the well-known affinity of boronic fragments towards carbohydrate residues. Solids were washed until no elimination of dye was observed. Table 1 lists the chemical composition of the active solids, calculated from thermogravimetric and elemental analysis. The guanidine content of **S2** was determined by analysis of the iodide counterion after dissolution in 34 wt% aqueous HF. The dye content of **S2-1** was calculated by colorimetric analysis after dissolution as for **S2**.

Table 1: Functionalization of **S2** and **S3**.

Solid	Binding molecules[a]/SiO_2	Solid	Dye/SiO_2[a]
S2	6.4×10^{-2}	**S2-1**	1.5×10^{-2}
S3	8.4×10^{-2}	**S3-2**	3.7×10^{-2}

[a] Molar ratios.

Figure 2b (inset) shows the ^{29}Si MAS-NMR spectrum of **S1**, in which the presence of two peak groups, assigned to the Q ($\delta = -105$, -97, -87 ppm) and T silicon centers ($\delta = -61$, -53 ppm), clearly indicates anchoring of the silane derivative to the silanol groups of the silica surface. Peak deconvolution gave a ratio of Q and T centers of 3:1, in good accordance with the chemical analysis. The successive functionalization process is displayed in Figure 3 which shows the IR spectra of the solids. In **S1**, bands due to OH vibrations have disappeared, and others related to the presence of NH_2 and CH_2 groups can be seen. In **S2**, bands assigned to the guanidinium groups are clearly observed (e.g., 1670 and 1600 cm^{-1}, assignable to the NH δ vibration). In the spectrum of **S2-1** bands due to dye vibrations (e.g., 1625 and 1404 cm^{-1}, assignable to the carboxylate groups) are observed. For solids **S3** and **S3-2** the bands due to the mannose residue and the azoic dye were also observed in the corresponding spectra.

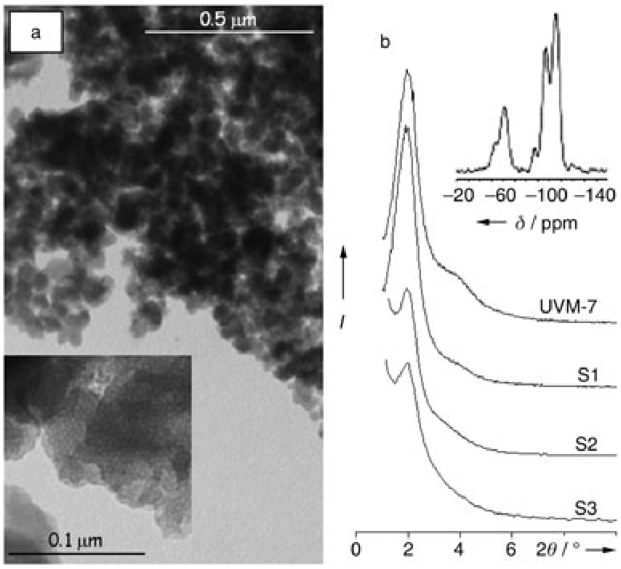

Figure 2. a) TEM micrograph of the guanidinium-functionalized solid after loading with methylthymol blue (**S2-1**). b) Powder XRD pattern of **S2** and **S3** compared with those of their precursors UVM-7 and **S1**. Inset: ^{29}Si MAS-NMR spectrum of functionalized **S1**.

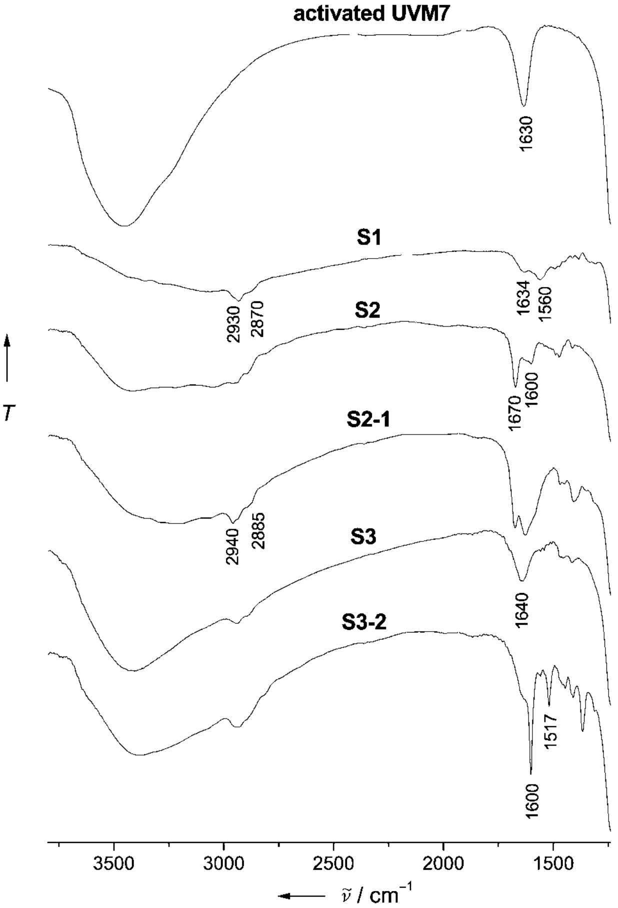

Figure 3. IR spectra of UVM-7, **S1**, **S2**, **S2-1**, **S3**, and **S3-2**. T = transmittance.

Structural characterization of the solids was carried out by TEM, powder XRD, and isothermal adsorption/desorption of N_2. Figure 2a shows a representative TEM micrograph of solid **S2-1** with the characteristic texture of UVM-7-type materials. Additionally, the inset clearly shows that the wormholelike MCM-41-type mesoporosity of the inorganic matrix is still present. Similar TEM images were obtained for the other materials. Figure 2b shows the variation of the powder XRD pattern of the UVM-7 matrix as it is functionalized to obtain **S2** and **S3**. The gradual intensity decrease is related to the loss of contrast as the amount of organic material anchored to the matrix increases. This increase in the organic fraction also influences the porosity of the obtained material. Table 2 summarizes the values obtained from the N_2 adsorption/desorption isotherms. A gradual decrease in specific surface area is associated with a concomitant decrease in pore size and volume. The changes are more pronounced for the small mesopores than for the large textural pores.

Guanidinium groups are known to undergo hydrogen-bonding interactions with carboxy groups, and therefore the **S2-1** ensemble was tested in displacement assays in water in the presence of the carboxylates citrate, succinate, lactate, malate, acetate, oxalate, propionate, formate, oxalacetate,

Table 2: Specific surface area and porosimetric data of **S1** and **S2**.

Solid	Specific surface area [$m^2 g^{-1}$]	Pore size[a] [nm]	Pore size[b] [nm]	Pore volume[a] [$cm^3 g^{-1}$]	Pore volume[b] [$cm^3 g^{-1}$]
S1	870	2.67	41.1	0.72	0.68
S2	377	2.1	35.4	0.24	0.58
S2-1	156	<1.7	18.2	0.08	0.21

[a] MCM-41-type pores. [b] Textural pores.

tartrate, maleate, malonate, glutarate, adipate, pimelate, and phthalate. The ensemble was suspended in water at pH 7.5 (HEPES 0.01 M) in the presence of a certain carboxylate, and after 10 min the mixture was filtered and the absorbance at 604 nm (visible band of dye **1**) measured. The results are summarized in Figure 4. A citrate-selective response was found that indicates that the binding pockets in **S2** are capable of recognizing this anion due to favorable coordination with respect to other carboxylates. Figure 4 also shows the calibration curve with citrate. No changes were observed in the presence of inorganic anions such as phosphate, sulfate, nitrate, carbonate, chloride, iodide, or bromide.

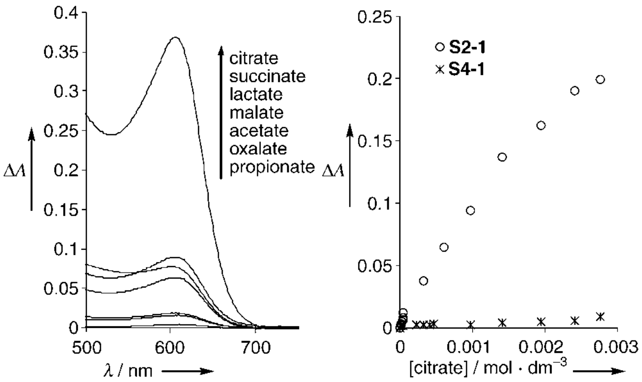

Figure 4. Left: Vis spectra (after filtering) of **S2-1** ensembles in the presence of different carboxylates. As examples, the responses to citrate, succinate, lactate, malate, acetate, oxalate, and propionate are shown. No significant response was found for other carboxylates. Right: citrate calibration curves for **S2-1** and **S4-1**. A detection limit for citrate of about 2×10^{-5} mol dm^{-3} was found for **S2-1**.

To test the effect of the nanoscale pores, we prepared **S4**, a similar material to **S2** but obtained from fumed silica (200 $m^2 g^{-1}$), which lacks the homogeneous porosity characteristic of mesoporous solids (**S4**: 1.5×10^{-2} guanidinium molecules per SiO_2, **S4-1**: 3.6×10^{-3} dye molecules per SiO_2; both values as molar ratios). Studies with citrate and **S4-1** (see Figure 4) showed a very poor response compared to that of **S2-1**. Despite the similar functionalization of **S2** and **S4**, their responses are remarkably different. This suggests that the presence of cylindrical-shaped pores in **S2** could favor spatial proximity between guanidinium subunits and thus enhance the coordinating ability of the binding groups in the nanoscale pockets towards citrate. This enhanced response was not observed for the nonporous surface of **S4**.

To further study the utility of the concept shown in Scheme 1, colorimetric sensing of borate in water/acetonitrile (80/20 v/v) was developed by using the **S3-2** ensemble. The ensemble was suspended at neutral pH in the presence of borate, and after 5 min the mixture was filtered and the band at 455 nm (due to release of **2** into the solution) measured. Dye displacement was observed in the presence of borate due to selective borate coordination with the diol groups in the surface of **S3**. Dye displacement is selective for borate (see Figure 5) and it is, as far as we know, the first chromogenic system based on supramolecular concepts for sensing the environmentally relevant borate anion.

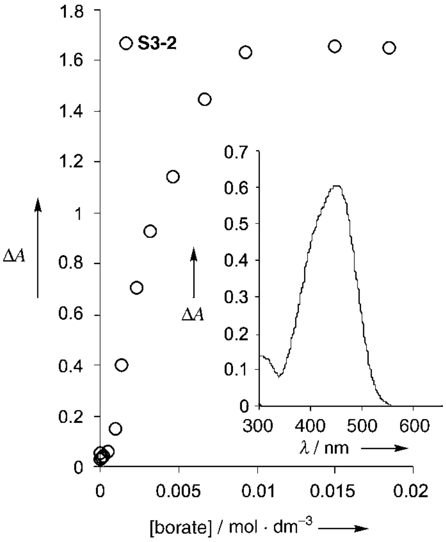

Figure 5. Borate calibration curve for **S3-2** (detection limit ca. 6×10^{-5} mol dm^{-3}). Inset: Vis spectrum (after filtering) of **S3-2** in the presence of borate and a mixture of different anions typically present in water (chloride, phosphate, sulfate, carbonate, etc.).

In summary, we have shown that functionalized mesoporous solids can act as binding pockets in anion-recognition processes. In particular, we have developed solids for displacement colorimetric assays in water for the selective detection of citrate and borate. We believe these findings can open new perspectives in the use of solids as hosts in solid-state/supramolecular chemistry concepts. The possibility of using a large number of mesoporous materials functionalized with different binding sites makes the approach suitable for the design of new chromo/fluorogenic probes for target guests.

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