

Hybrid materials with nanoscopic anion-binding pockets for the colorimetric sensing of phosphate in water using displacement assays†

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Mesoporous amino-functionalised solids containing certain dyes have been used as suitable anion hosts in displacement assays for the colorimetric signalling of phosphate in water.

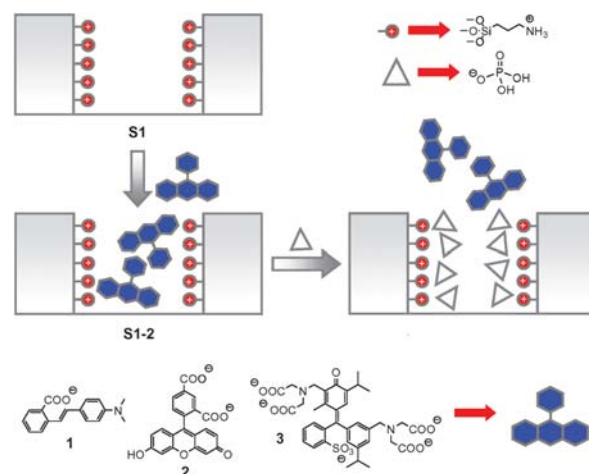
One recently suggested procedure to develop new supramolecular concepts is to attach molecular/supramolecular entities to pre-organised nanoscopic structures. These hybrid systems show cooperative functional behaviours that result in chemical amplification processes.¹ In this field, recent research for the development of smart sensory materials has been reported.² This approach allows in a simple way to create hybrid sensing ensembles that can signal analytes for whose selectivity is hard to achieve by conventional methods or may require extensive synthetic chemistry in order to prepare selective receptors. Also the design of hybrid solids with certain surface features (*e.g.* the presence of mesopores) has been reported to provide new unpredicted functional synergic effects.³ Following our interest in the development of sensing systems using hybrid materials⁴ we report herein how the combination of supramolecular and nanoscopic solid-state concepts allows the development of a simple signalling protocol for chromogenic detection of phosphate in aqueous solutions.

Selective chromogenic phosphate receptors are very rare and very few examples displaying colorimetric sensing features for this anion in pure water based on coordination/supramolecular ideas have been described. In one of them a dinuclear Zn(II) complex with H-bmpm (2,6-bis(bis(2-pyridylmethyl)aminomethyl)-4-methylphenol) forms an ensemble with Pyrocatechol Violet (1 : 1 stoichiometry) that changes its colour selectively in the presence of phosphate *via* phosphate coordination with the Zn complex and release of the pyrocatechol dye.⁵ The other reported example uses a sensing ensemble formed between Pyrocatechol Violet with YbCl₃.⁶ Addition of phosphate to the formed complex results in a yellow coloration of the solution due to YbCl₃-phosphate coordination and release of the dye. Also very recently and following a remarkable approach, the effective sensing of phosphate and other biologically important phosphate-derivatives in water has been successfully achieved by the use of arrays of relatively simple chromogenic receptors embedded in olyurethane membranes.⁷

These examples are all molecular-based. In contrast, we have followed here a different protocol relying on the use of nanoscopic hybrid “binding pockets” as mimicking systems of active-site cavities in biological systems. In fact recent interesting examples dealing with the functionalization of nanoscopic pockets on solid supports with suitable dyes have been used for the development of colorimetric probes in water for certain metal cations⁸ and anions⁹ with very fine results. We believed that this biomimetic approach using simple displacement assays¹⁰ may allow us to design probes for optical signalling of target guests.

The designed protocol for the colorimetric signaling of phosphate is shown in Scheme 1. Suitable nano-sized pores from a MCM41-type material are functionalized with simple amine binding sites in one step. Then the functionalized solid is loaded with a certain dye able to give binding interactions with the coordination sites. This is the signalling reporter. In the sensing protocol, the target analyte is preferentially bonded by the nanoscopic hybrid binding pocket, inducing the delivery of the dye to the solution resulting in the colorimetric detection of the guest.

Inspired by this concept the “host” 2.4 nm pore diameter mesoporous solid **S1** containing nanoscale cavities was prepared and loaded with suitable dyes as signalling reporters. In order to enhance accessibility onto the pores we used as starting material the mesoporous MCM41-type UVM-7 derivative which is characterized by their nanometric particle size and bimodal pore system.¹¹ This mesoporous material was



Scheme 1 Protocol for phosphate signalling in water using mesoporous solids containing nanoscopic binding pockets and suitable dyes.

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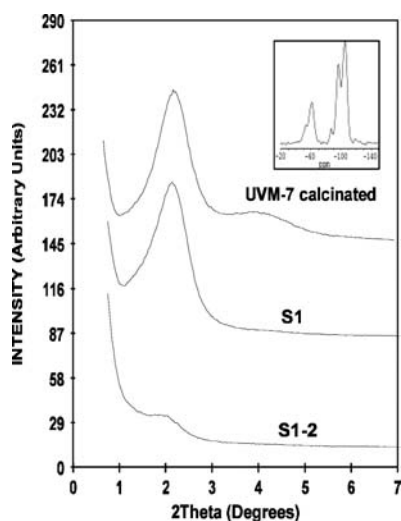


Fig. 1 X-Ray powder diffraction for the calcined UVM-7, **S1** and **S1-2** materials. The inset shows the ^{29}Si MAS NMR spectrum of the amine-functionalised solid **S1**.

first made to react with 3-aminopropyltriethoxysilane to yield solid **S1**. To introduce the signalling units into the pores, solid **S1** was further loaded with three different dyes (*i.e.* Methyl Red, **1**, Carboxyfluorescein, **2** and Methylthymol Blue, **3**) at room temperature in water at pH 7.5 for 24 h to yield solids **S1-1**, **S1-2** and **S1-3** (see Scheme 1) that were isolated by filtration and washed until no elimination of the dye to the naked eye was observed. The amines in solid **S1** at neutral pH in water are expected to be partially protonated and therefore binding with the anionic carboxylate-containing dyes **1-3** occurs *via* hydrogen bonding and electrostatic interactions. The final **S1** solid has an amine content of 0.175 mol amine per mol of SiO_2 , whereas typical dye loading for different prepared solids was in the 0.02–0.1 dye/ SiO_2 mol/mol range.

Characterization of the materials by XRD and TEM techniques has been carried out. Fig. 1 shows the XRD patterns for the UVM-7, the amine-functionalised solid **S1** and the final carboxyfluorescein-loaded **S1-2** sensory material. The X-ray powder pattern of UVM-1 and **S1** are very similar whereas the low-angle (100) reflection decreases in intensity for **S1-2** due to a decrease in contrast as a consequence of the larger filling of the pores by the organic dye. Moreover, the existence of the d_{100} peak and the presence in the TEM images of the typical mesoporous structure (see Fig. 2) strongly evidence that the functionalization with the amine and the further loading with the dye does not affect the mesoporous UVM-7 type scaffolding. The ^{29}Si MAS NMR spectrum of functionalised **S1-2** solid is shown in the inset. It displays the presence of two groups of signals that correspond to T-silicon (–61 and –53 ppm) and Q-silicon (–105, –97 and –87 ppm) centres, indicative of the

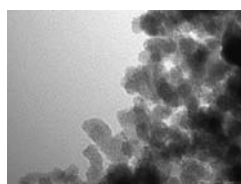


Fig. 2 TEM image of solid **S1-2** showing the nanometric particle size.

condensation of the aminosilane derivative with the silanol groups on of the silica surface in the preparation of **S1**. Spectrum deconvolution gave an approximate relation between Q and T centres of 3 : 1 in good accordance with chemical analysis data (see ESI† for further characterization details).

Amines have been extensively used as suitable groups for the design of receptors able to bind anionic guests. Solids **S1-1**, **S1-2** and **S1-3** contain nanoscopic binding pockets functionalised with anion coordination sites (amines) and different dyes. In a typical experiment 30 mg of the corresponding solid were suspended in water at pH 7.5 (HEPES 0.01 mol dm^{-3}) in the presence of a certain anion and after 10 min the mixture was filtered and the absorbance at the corresponding visible band measured (426, 490 and 605 nm for Methyl Red (solid **S1-1**), Carboxyfluorescein (solid **S1-2**) and Methylthymol Blue (solid **S1-3**), respectively). The anions fluoride, chloride, bromide, iodide, phosphate, sulfate, acetate and nitrate were studied as sodium or potassium salts. The results are summarized in Fig. 3.

Addition of phosphate to the functionalised solid suspension resulted in a remarkable development of colour, especially for **S1-2**. Addition of similar anions such as sulfate also induced some colour but to a remarkably lesser extent than phosphate. The solutions remained completely colourless for the **S1-2** and **S1-3** sensory ensembles in the presence of fluoride, chloride, bromide, iodide, acetate and nitrate, whereas **S1-1** also shows some response to acetate.

A phosphate-selective response was found indicating that the binding pockets in **S1-2** and **S1-3** are capable of recognising phosphate due to favourable coordination when compared with other anions typically present in water. The remarkable phosphate selective behaviour can be explained in terms of preferential formation of phosphate complexes with the tethered amines in the pores. The observed conduct is in agreement with the polyammonium-anion complex formation trend in water that usually follows the order phosphate > sulfate \gg nitrate \sim halide ions.¹² Thus, a suitable design of the solid coupled with this phosphate binding favourable feature results in a colorimetric selective response to this anion in pure water. Additionally, **S1-2** shows the best response among the solids tested in terms of sensitivity. Thus absorbances up to values of *ca.* 2 can be easily be observed using **S1-2** in the presence of

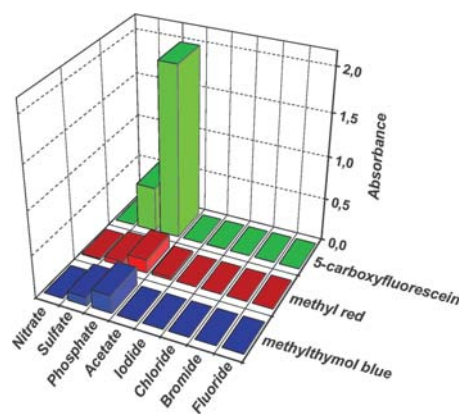


Fig. 3 Colorimetric response of solids **S1-1**, **S1-2** and **S1-3** to the presence of certain anions in pure water at pH 7.5 (HEPES 0.01 mol dm^{-3}), $C_{\text{anion}} = 1 \times 10^{-3}$ mol dm^{-3} .

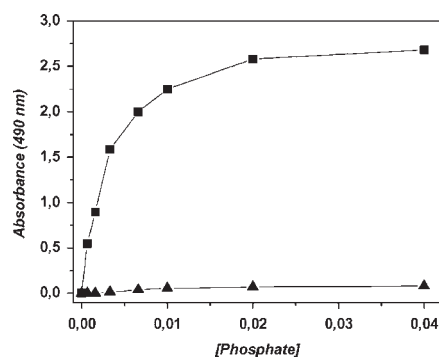


Fig. 4 Calibration curve for solids **S1-2** (■) and **SG-2** (▲) at pH 7.5 (HEPES 0.01 mol dm⁻³) in the presence of phosphate.

phosphate. By contrast, under similar conditions **S1-3** shows a poorer response most likely because the Methylthymol Blue dye (a trianion) in **S1-3** forms quite strong complexes with the anchored amines. Consequently, as phosphate must be present as a mixture of mono- and di-anionic species (HPO_4^{2-} and H_2PO_4^-) at neutral pH, the trianion is poorly displaced. In fact we have observed under competitive conditions that Methylthymol Blue is preferentially included with respect to 5-Carboxyfluorescein in the nanoscopic pockets of **S1** indicating that the former dye binds more tightly with the ammonium groups (see ESI† for details). The solid **S1-1** shows a poor response presumably because the monocarboxylate dye Methyl Red is weakly adsorbed at the mesopores and it is released in the washing stage during its preparation.

An additional important aspect in this research was to find out the effect that the nanoscopic pockets have in the sensing behaviour of these hybrid materials. Therefore as an additional study, we prepared a material similar to **S1-2** but using non-porous silica (silica gel) as solid support. This new solid (**SG-2**) contains anchored amine groups and a certain amount of adsorbed Carboxyfluorescein, *via* interaction with the amines at the surface, but lacks the 3D mesoporous structure in **S1-2**. Studies of the interaction of **SG-2** with phosphate and other anions showed no response (see Fig. 4). This result shows the effect that the mesoporous 3D organized surface has in the sensing protocol. The presence of nanoscopic binding pockets in **S1** most likely induces some spatial proximity between several ammonium subunits forming a suitable “binding pocket” within the pores that favours coordination first with the dye and then with phosphate. This synergic effect is not apparently observed when using locally flat surfaces (**SG-2** material).

The response of the sensing material **S1-2** and **SG-2** as a function of the phosphate concentration in water at neutral pH is illustrated in Fig. 4 which plots the absorbance at 490 nm after the displacement protocol described above. The results reveal that the delivery of the dye from **S1-2** to the solution is related with the phosphate–solid interaction and therefore proportional to the amount of phosphate in water. A detection limit lower than 1×10^{-4} mol dm⁻³ was calculated.

In summary, we have shown that simple functionalised mesoporous solids containing nanosized coordinating pockets also containing commercially available dyes can act as suitable sensory materials for the chromo-fluorogenic detection of

anions of environmental importance such as phosphate. The approach allows with a minimum effort to test a large number of combinations and may be of application to a wide range of different guests. The sensing protocol is undemanding and opens the possibility of developing new selective signalling systems by combination of simple coordinating groups and the non-covalent anchoring of suitable dyes in similar hybrid systems.

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