Chromo-fluorogenic sensing of pyrophosphate in aqueous media using silica functionalised with binding and reactive units†

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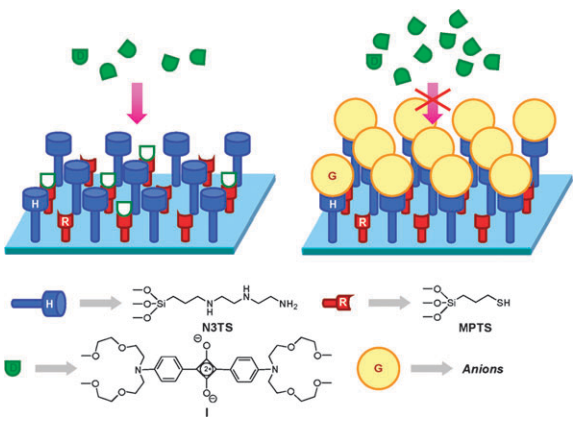
A chromogenic chemosensor for selective pyrophosphate sensing in aqueous environments has been developed using silica functionalised surfaces.

The attachment of molecular entities following supramolecular concepts on pre-organized solid structures has recently resulted in a number of new functional enhanced chemical effects.[1] Within this field, the functionalization of solids with certain groups to enhance recognition or switching is particularly appealing. Additionally, related with signalling, this approach has resulted, through the use of relatively simple subunits, in the development of hybrid sensing ensembles that effectively compete in selectivity and applicability with more classical conventional sensing methods. Following our interest in the study of supramolecular functional aspects that arise from the covalent grafting of organic molecules to solid supports,[2] we report herein the design of a new chromo-fluorogenic protocol using binding sites and reactive subunits anchored into a siliceous support. As a proof-of-the-concept the new sensing scheme has been applied to the chromo-fluorogenic detection of pyrophosphate in aqueous environments.

The new chromogenic signalling paradigm is shown in Scheme 1. It involves the use of a suitable support functionalised with two distinctive groups. One is a reactive unit (R) that is known to react with a certain dye (D) inducing bleaching, whereas the other anchored group is a host site (H) able to coordinate certain target guests (G). In the absence of G, the groups R will react with the dye resulting in a bleaching of the solution (“off state”). On the contrary, the presence of the guest G results in a control of the reaction kinetics between R and D and eventually in a complete reaction inhibition (“on state”). This protocol is reminiscent of the so-called ion channel sensors (ICSs). The principle of operation of ICSs, first reported by Umezawa,[3] consists of a conducting solid (an electrode) and a layer of anchored molecular binding sites. Binding of target guests to the coordinating layer modulates the accessibility of a redox group (redox marker) to the electrode surface resulting in an overall guest-controlled switching functional effect and electrochemical detection. Based in this concept some sensors have been reported for a range of species from simple metal ions[4] to complex guests.[5] However, as far as we know, from the literature it is apparent that studies similar to those shown in Scheme 1 for the design of chromo-fluorogenic probes have never been reported. Moreover, we believe this might be a helpful new paradigm for the development of, easy to prepare, chromo-fluorogenic signalling systems. For instance, the nature of the reactive unit, the size and charge of the dye and the anchored binding groups can be easily changed, giving rise to tailor-made functional solids that can eventually show enhanced colorimetric selective response towards target guests.

The specific development of a chromo-fluorogenic probe requires the choice of a suitable signalling reaction (the pair “R”, “D” in Scheme 1) and the selection of a binding site–guest system (the pair “H”, “G” in Scheme 1). In this work, and as a proof-of-the-concept of the potential use of the protocol shown in Scheme 1, we have selected as signalling pair a thiol group (“R”) and a squaraine dye (“D”) based on the well-known thiol–squaraine reactivity.[6] This is a selective reaction that occurs *via* nucleophilic attack of the thiol group to the electron deficient central four-membered ring of the squaraine scaffolding resulting in a rupture of the electronic delocalization and a bleaching of the blue squaraine solutions. This is a very rapid reaction that occurs at room temperature in a relatively wide pH range. The squaraine derivative **I** was synthesized following literature procedures.[7] For the signalling protocol a water–CH_3CN (90 : 10 v/v) mixture was selected due to the poor solubility of squaraine **I** in pure water. As



Scheme 1 Idealised representation of the sensing paradigm for the chromogenic recognition of anions. In the absence of anions the dye (D) reacts with R inducing the bleaching of the solution (left). In the presence of certain anions (G) the coordination with the host site (H) induced the inhibition of the reaction between R and D (right).

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binding site–guest system polyamines and anions have been selected, respectively. Protonation of the amines at a certain pH is expected to result in the formation of the corresponding complexes with certain anionic species that would control the reactivity/accessibility of the squaraine derivative from the solution to the thiol groups anchored on the surface. In fact, amines, especially when transformed to ammonium groups by protonation, are suitable anion coordination hosts usually *via* a combination of hydrogen bonding interactions and electrostatic forces.[8] The host and reactive unit components have to be additionally anchored onto a suitable support. For preliminary studies, we chose here fumed silica (a support with a specific surface area of 200 $m^2\ g^{-1}$ and an average particle size of 0.014 μm) as simple yet suitable inorganic structure due to its inertness and ease of functionalization.

The functionalised solid **S1** was prepared using the following procedure: the starting solid (fumed silica activated with HCl) was suspended in toluene anhydrous and the mixture was heated up to 140 °C in order to eliminate water by azeotropic distillation. Then, a mixture of mercaptopropyltrimethoxysilane (**MPTS**, 14.06 mmol) and 3-[2-(2-aminoethylamino)ethylamino]propyltrimethoxysilane (**N3TS**, 0.94 mmol) were added to the silica suspension. The final material was then filtered, washed with toluene and dried at 70 °C for 12 h. Also, and by the same procedure, the model solid **S1–SH** consisting of silica functionalised only with thiol groups was synthesised. Solid **S1** was characterized using standard procedures. The content of thiol and polyamine were determined by elemental analysis and thermogravimetric measurements and amounts to 0.089 and 0.343 mmol/g SiO_2, respectively.

The reactivity of the signalling dye D (squaraine **I**) with the reactive centre R (thiol groups) was studied at different pH values and in the presence of certain inorganic anions. Although, studies with solid **S1** were carried out in a range of pH values, only results at pH 3, 5 and 7 are shown here for the sake of clarity. Too basic pH values were avoided in this study in order to stay away from the possible damage of the siliceous matrix at high concentration of hydroxide anions. Also very acidic or basic solutions have been reported to result in a degradation of the squaraine dye. As is detailed below the reactivity of the squaraine **I** and the thiols anchored on the surface of **S1** can be controlled by both the pH and by the presence of certain anions.

The reactivity of **S1** with the squaraine **I** has been studied in the presence of chloride, perchlorate, nitrate, sulfate, phosphate and pyrophosphate. Studies were carried out with suspensions of **S1** in water–acetonitrile (90 : 10 v/v) mixtures containing an amount of the corresponding anion. Then **I** was added, after 15 min the suspension was filtered and the absorbance of the squaraine in the solution measured. From the studies carried out, the results at pH 5 are the most remarkable at which a selective response was clearly observed. Fig. 1 shows the response of solid **S1** at pH 5 in the presence of increasing concentrations of different inorganic anions.

The figure shows that at this pH the anion pyrophosphate (in $HP_2O_7^{3-}$ form) is able to inhibit the reaction of the squaraine **I** with the thiol, whereas this inhibition does not happen in the presence of the remaining anions (phosphate, sulfate, nitrate, perchlorate or chloride). As can be seen in the figure the overall result is a highly selective colorimetric signalling of pyrophosphate. Additionally, by using fluorescence measurements (the quantum yield of **I** in water is 0.015, λ_{exc} = 643 nm and λ_{em} = 666 nm) a detection limit as low as *ca.* 1.0×10^{-7} mol dm^{-3} for pyrophosphate can be reached following this simple procedure.

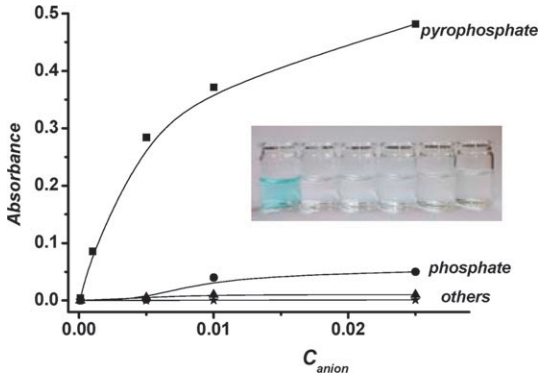

Fig. 1 Absorbance at 643 nm (squaraine band) *vs.* concentration of pyrophosphate, phosphate, sulfate, nitrate, chloride and perchlorate at pH 5 for solid **S1** in water–acetonitrile (90 : 10 v/v). Inset: Photograph showing the colorimetric detection of pyrophosphate. The correspondent anions (5.0×10^{-3} mol dm^{-3}) were added to suspensions of solid **S1** and **I** in water–acetonitrile (90 : 10 v/v) mixtures. After 15 min the suspension was filtered. From left to right: pyrophosphate, phosphate, sulfate, nitrate, chloride and perchlorate.

The kinetics of the reaction between squaraine **I** and the thiol group at pH 5.0 for different concentrations of pyrophosphate anion has been studied. This reaction between squaraine and thiol presents a second-order kinetics as could be clearly seen in Fig. 2. A clear dependence of the kinetic of the squaraine bleaching reaction is observed in the presence of increasing amounts of pyrophosphate.

This highly selective colorimetric signalling of pyrophosphate with respect to other inorganic anions in mixed aqueous solutions, based on a control of dye transport (squaraine) from the solution to the solid surface, is quite unusual and is hardly achievable using classical chemosensors. In fact, there are very few examples of chromogenic pyrophosphate sensing in water or mixed aqueous solutions.[9]

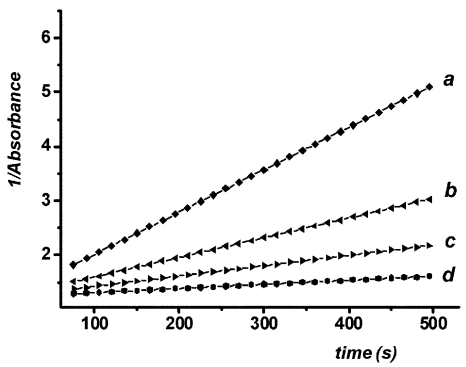

Fig. 2 Inverse of the absorbance at 643 nm *vs.* time for the reaction between the anchored thiols in **S1** and squaraine at pH 5.0 for different concentrations of pyrophosphate (a, 0.001 mol dm^{-3}; b, 0.0025 mol dm^{-3}; c, 0.005 mol dm^{-3}: d, 0.01 mol dm^{-3}).

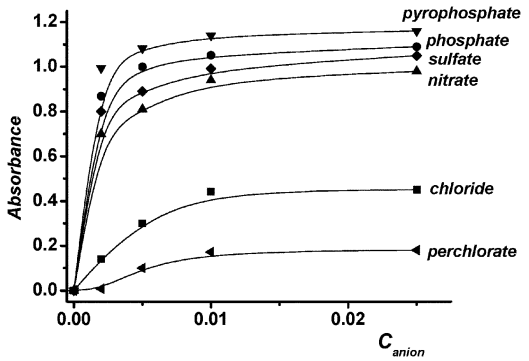

Fig. 3 Absorbance at 643 nm (squaraine band) *vs.* concentration of pyrophosphate, phosphate, sulfate, nitrate, chloride and perchlorate at pH 3 for solid **S1** in water–acetonitrile (90 : 10 v/v).

We believe that this observed behaviour must be attributed to the formation of strong pyrophosphate complexes with the tethered polyamines at pH 5. In fact this is in agreement with the polyamine/polyammonium literature that reports that polyamines form very strong complexes with phosphate-derivatives. The colorimetric response is additionally controlled by changes in the pH. This pH effect (see below) is dependent on the degree of protonation of the amines as a function of the pH.[10] From a simple titration of acidic solutions of **S1** with sodium hydroxide percentages of protonation of the anchored amines on **S1** of 23, 59 and 81% for pH 7, 5 and 3, were calculated, respectively. Therefore at pH 3 the polyamines are much more protonated than at pH 5 and are prone to form more and stronger complexes with anionic species. As a result of this enhanced coordination effect at pH 3 the anions pyrophosphate (in the form of $H_2P_2O_7^{2-}$), phosphate (in the form of $H_2PO_4^-$), sulfate and nitrate are able to completely inhibit the reaction of squaraine **I** with the thiol groups anchored on the silica surface, whereas this inhibition is less remarkable with perchlorate and chloride (see Fig. 3). On the other hand, at pH 7 polyamines are less protonated, and their interactions with anions, both by strength and number, are drastically reduced, therefore the squaraine **I** reacts very rapidly with the thiol groups in the presence of all the anions studied. In this case no changes in the apparent reaction kinetics are observed even at concentrations up to 3×10^{-2} mol dm^{-3} of the anionic guests (see ESI†).

Finally, we were particularly concerned with the possibility that the observed effect was not caused by the amine-containing ensemble but due to some simple change in the reactivity of the squaraine dye with the thiol groups anchored on the silica surface as a function of the pH or in the presence of certain anions. To eliminate this possibility the response of solid **S1–SH** was tested. This solid does not display pH- or anion-controlled effects and a very rapid pH-independent decoloration of the dye (due to a rapid reaction with the SH groups) is found in aqueous solution indicating that the control of the reactivity observed in Fig. 1 is due to coordination of the protonated amines (the coordination site H) with the anionic guests (G, see Scheme 1).

In summary, we have shown here for the first time and as a proof-of-the-concept how hybrid system (*i.e.* simple silica surface functionalised with a thiol reactive unit and a polyamine) can be used for the design and development of new protocols for anion colorimetric signalling. The possibility of easily changing the reactive unit, the dye and the binding sites opens the opportunity to develop new and advanced functional sensing solids for the selective colorimetric or fluorimetric signalling of target guests.

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