Chromogenic silica nanoparticles for the colorimetric sensing of long-chain carboxylates†

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Silica nanoparticles functionalized with chromogenic spirobenzopyran and thiourea subunits show selective colour changes in the presence of certain long-chain carboxylates.

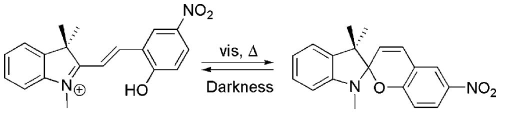
In recent years the blending of supramolecular and material chemistry has lead to the preparation of novel hybrid systems showing synergic effects that are hardly achievable within molecular-based systems or solids alone.[1] In this area prominent examples involving the combination of supramolecular ideas and nanoscopic scaffoldings have recently been reported.[2] One of the fields in which these hybrid materials is ensured to have promising applications is related to the development of optical chemical sensors.[3] Among different nanoscopic scaffoldings the use of quantum dots[4] and gold or silica nanoparticles is especially appealing due to their easy preparation, straightforward surface functionalization and high stability in water.[5] For instance, shift of the plasmon band of crown ether-modified gold nanoparticles due to aggregation processes has recently been used for the selective recognition of metal cations in water.[6] Also, silica nanoparticles functionalized with organic fluorophores have been used for the fluorescent recognition of certain metal cations.[7] Despite these encouraging examples, the use of nanoparticle-based hybrid sensors for the specific optical recognition of anions has been much less explored and only certain examples dealing with the use of colour changes induced by anion-induced aggregation processes in gold nanoparticles have been described.[8] In contrast, as far as we know, silica nanoparticles have never been used in the development of hybrid materials for optical anion recognition.

Following our interest in the search for new concepts for anion sensing, we focused our attention towards silica nanoparticles and the use of the spiropyran photochrome as a suitable signalling unit. The latter is a well-known bistable system than can be transformed reversibly between two different molecular entities; when the spirocyclic form is irradiated with UV light, or put in the dark, it isomerizes to the merocyanine form which is positively charged at neutral pH or a zwitterion at high pH (see Scheme 1).[9] Additionally, the spyropyran–merocyanine transformation can be followed by changes from the reddish colour of the merocyanine to the pale colour of the cyclic form.[10] This particular behaviour of the spiropyran–merocyanine couple has been widely used for the preparation of light-switchable systems.[11]

However, we have focused here on a less common approach to achieve the merocyanine–spiropyran transformation that entails changes in the environment polarity; *i.e.* the spirocycle is formed favourably in highly hydrophobic environments whereas the more polar merocyanine derivative is stabilized in hydrophilic media.[12] Following this concept, a solid **N1** was prepared to assess as a proof-of-concept the possibility of developing new chromogenic sensing protocols based on a merocyanine–spiropyran anion-controlled transformation. The sensing approach is schematically shown in Scheme 2. It involves the independent anchoring of anion binding sites (thiourea) and the merocyanine signalling groups in close proximity on the surface of a silica nanoparticle. The signalling event is based on the concept that coordination of anions with different hydrophobicity (for instance certain carboxylates, see below) would modify the merocyanine–spiropyran ratio *via* an anion-induced polarity modulations near the signalling groups, therefore resulting in a colour modulation (see Scheme 2).

The binding site and signalling units anchored to the silica nanoparticles for the preparation of **N1** are shown in Scheme 3. The spirobenzopyran derivative 1′-(3-triethoxysilanpropyl)-3′,3′-dimethyl-6-nitrospiro[2*H*-1]benzopyran-2,2′-indoline (**1**) was synthesized in two steps[10] whereas the *N*-phenyl-*N*′-[3-(triethoxysilyl)propyl]thiourea (**2**) was prepared by direct reaction between phenylisothiocyanate and aminopropyltriethoxysilane.

These two compounds were attached to the nanoparticle surface through the following procedure: 5 mL of a 30% suspension of Ludox silica nanoparticles AS-30 (20 nm average diameter) were added to a solution containing 20 mL of acetic acid, 40 mL of ethanol, 0.2 mmol of **1** and 1.2 mmol of **2**. The crude reaction was heated at 80 °C for 24 h, the ethanol was evaporated and then the acetic acid solution neutralized with a saturated solution of sodium hydrogen carbonate. The



Scheme 1 Merocyanine–spiropyran transformation.

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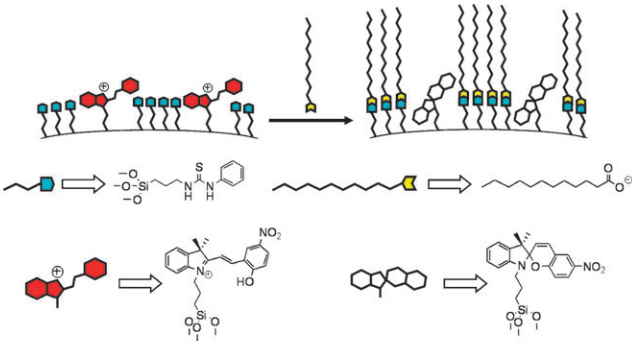


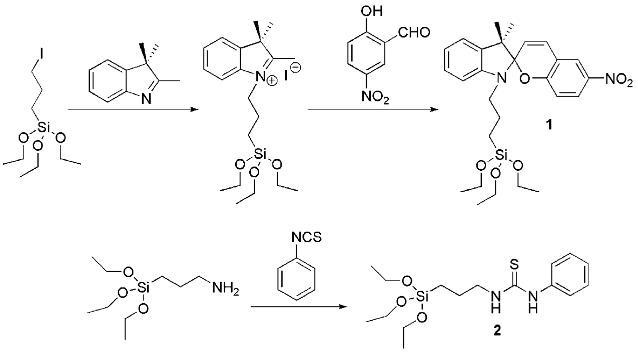
Scheme 2 Signalling protocol. Left, the anion binding sites (thiourea) and the signalling unit that is in its stable form in water; *i.e.* merocyanine form. Right, coordination of large carboxylates on the binding site induce remarkable changes in polarity around the signalling group inducing cyclization to the spyrocyclic form and colour modulation from pink-red to pale yellow.

functionalized nanoparticles (**N1**) precipitated and were isolated by filtration, washed with water and acetone and dried at 70 °C. Also, and by a similar procedure, nanoparticles functionalized only with the spiropyran derivative **1** were synthesized (**N2**) as a model derivative for the elucidation of the role played by the anionic binding site.

N1 and **N2** materials were characterized by following standard techniques. From thermogravimetric, elemental analyses and X-ray absorption microanalyses, contents of 16 mmol of thiourea/mol SiO_2 and 4 mmol of spiropyran/mol SiO_2 in **N1** were found. The spiropyran content in **N2** was found to be 11 mmol/mol SiO_2. Additionally **N1** and **N2** show a very homogenous particle size of around 20 nm (see TEM image in the ESI†). As a rough estimate, each coated nanoparticle bears up to 1500 attached subunit molecules. The average distance between two subunits amounts to 10.3 Å.

In a first step it was demonstrated that the polarity-induced merocyanine–spiropyran switching still remains after the grafting of the signalling unit onto the nanoparticles. This can be seen in Fig. 1, which shows the colour of precipitated **N1** nanoparticles after being in contact with water (left) or hexane (right). This colour modulation shows that the red merocyanine form in polar media (water) is readily transformed into the corresponding pale yellow spirocyclic form in a lipophilic environment (hexane).

Stimulated by these favourable features, the potential sensing behaviour of **N1** in water (HEPES 0.001 mol dm^{-3} at pH 7) was studied against a family of carboxylates of increasing hydrophobicity, *i.e.* acetate, butanoate, hexanoate, octanoate, decanoate and dodecanoate. In a typical experiment, 20 mg of **N1** were added to 2 ml of the respective carboxylate solution ($C_{\text{carboxylate}} = 1 \times 10^{-3}$ mol dm^{-3}) in HEPES (1×10^{-3} mol dm^{-3} at pH 7) and then the nanoparticles were suspended by using a rotor–stator system (Ultra-Turrax) for 4 min and then ultrasonicated at 25 °C.



Scheme 3 Synthesis of compounds **1** and **2**.

Fig. 1 Changes in the colour of nanoparticles **N1** after being in contact with water (left) and with hexane (right).

As an example, Fig. 2 shows the different colours of precipitated **N1** nanoparticles after their suspension in aqueous solutions containing hexanoate or dodecanoate ($C = 1 \times 10^{-3}$ mol dm^{-3}). As can be seen in Fig. 2 there is a gradual change in colour from red, for solid **N1** alone in water, to pale red in the presence of hexanoate and pale yellow in the presence of dodecanoate. This behaviour clearly resembles that shown by **N1** nanoparticles in water or hexane suspensions (*vide ante*) and is in accordance with carboxylate coordination to the binding sites (thiourea) that results in a polarity-induced merocyanine-to-spirobenzopyran transformation.

For the other carboxylates tested a gradual colour change from magenta/red to light pink clearly linked with the length of the alkyl chain was observed. The polarity-controlled mechanism was assessed by the fact that the addition of certain inorganic anions (fluoride, chloride, bromide, iodide, cyanide, sulfate, phosphate and nitrate) induced negligible colour changes in water–**N1** suspensions. In order to quantify the colour modulation upon addition of different carboxylates, the colours of the water–**N1** suspensions were measured using an optical fibre UV-visible spectrophotometer and characterized on the CIE 1976 (L, a, b) colour space.[13] This system is one of the most complete colour modes conventionally used to describe visible colours to the human eye. It consists of three coordinates that represent the lightness of the colour (L) and its position between magenta/green and yellow/blue (a and b, respectively). In this system each colour is a point in three dimensional space and the relative difference between any two colours is given by ΔE which represents the Euclidean distance between them.

The difference between colours as ΔE values of **N1** and **N1** nanoparticles upon addition of the respective carboxylate are plotted in Fig. 3. As can be seen there is a gradual increment in the value of ΔE upon the increase in the length of the alkyl chain of the carboxylate. Changes in ΔE correlated well with the coordination of the carboxylates to the thiourea moieties and therefore formation of a self assembled monolayer of different degrees of hydrophobicity around the signalling reporter.

The interaction of **N1** nanoparticles with hexanoate and dodecanoate was studied in more detail using elemental and

Fig. 2 Changes in colour observed in **N1** nanoparticles in the presence of certain carboxylates. From left to right: no carboxylate, hexanoate and dodecanoate.

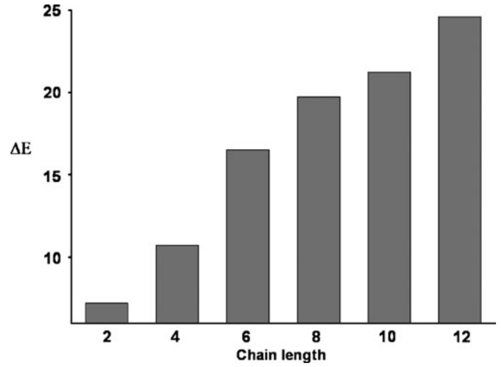


Fig. 3 ΔE values for **N1** nanoparticles in the presence of carboxylates in water ($C_{\text{carboxylate}} = 1 \times 10^{-3}$ mol dm^{-3}, HEPES, pH 7).

thermogravimetric analysis after the interaction of **N1** with neutral solutions of these carboxylates. From these analyses it was evident that both carboxylates tend to coordinate on the surface of the **N1** nanoparticles. For instance, contents in the range of *ca.* 14–17 mmol of carboxylate/mol SiO_2 were found to be anchored to the **N1** nanoparticles. These values are in good agreement with the thiourea content in **N1**, strongly suggesting the formation of a dense, highly hydrophobic monolayer around the signalling reporter that is responsible for the signalling event.

In order to elucidate if the colour changes observed with **N1** nanoparticles were only induced by the environment polarity a test with lauryl sulfate (a lipophilic anion) was carried out. Addition of lauryl sulfate solutions ($C = 1 \times 10^{-3}$ mol dm^{-3}) in HEPES (1×10^{-3} mol dm^{-3} at pH 7) to a stirred suspension of **N1** nanoparticles (20 mg in HEPES 0.001 mol dm^{-3} at pH 7) does not induce any noticeable colour change. This result points toward the relevance, not only of the environment polarity around the colorimetric probe generated upon co-ordination, but also of the chemical nature of the binding site. Thiourea binds carboxylates strongly whereas it is unable to coordinate with the sulfate group of the lauryl sulfate. The usefulness of this approach, towards the development of novel sensing systems, is remarkable because, additionally to the polarity control, the selectivity of the material could be tuned simply by changing the chemical structure of the binding site.

Finally, we were concerned with the possibility that the observed effect might be caused by simple interactions of the carboxylates with the silica surface. To eliminate this possibility the experiments were repeated using an aqueous suspension of the **N2** nanoparticles (*i.e.* functionalized with the signalling reporter but not containing thiourea groups). These nanoparticles do not show a response, showing the relevance that the presence of the thiourea groups has in relation to the development of the colour modulation and strongly suggesting that colour modulations are not due to carboxylate interaction with the nanoparticle silica surface, but most likely due to the polarity-induced effects described above.

In conclusion we have synthesized hybrid silica-based nanoparticles by anchoring independent spiropyran groups as signalling units and thiourea moieties as binding sites for the colorimetric signalling of carboxylates in pure water *via* polarity-induced colour modulation. Further studies with other carboxylates and supports are being carried out, seeking the applicability of this concept. The possibility of using different molecular entities sensitive to polarity changes combined with anion binding sites anchored on supports, makes the results we show here of interest in relation to the opening of new prospects in tailor-made materials for advanced chromo-fluorogenic sensing.

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