

Supporting Information

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

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1. Experimental procedures

2,3,3-trimethylindolenine, (3-iodopropyl)triethoxysilane, phenylisothiocyanate, (3-aminopropyl)triethoxysilane, 2-hydroxy-5-nitrobenzaldehyde, triethylamine, 30 % suspension of ludox silica nanoparticles AS-30 Colloidal Silica were purchased from Sigma-Aldrich and were used without any further purification. The solvents were absolute grade and were purchased from Scharlab. Anion stock solutions ($C_{anion} = 1 \times 10^{-3} \text{ mol dm}^{-3}$) were prepared in HEPES ($0.001 \text{ mol dm}^{-3}$ at pH 7). The buffer 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES, Sigma) was used as supplied by the manufacturers.

^1H and ^{13}C NMR spectra were recorded using a Varian Gemini 300 MHz NMR spectrometer. Chemical shifts are reported relative to internal Me_4Si . Multiplicity is given as usual. Thermogravimetric analysis were carried out in a Mettler Toledo TGA/SDTA 851°. Transmission Electron Microscopy (TEM) images of the particles were obtained with a Philips CM10 operating at 20 KeV. Samples for TEM were prepared by spreading a drop of nanoparticles solution in HEPES onto standard carbon-coated copper grids (200 mesh). SEM microanalyses were obtained using a JEOL 6300 with a detector WDS of Oxford Instruments. UV-Vis absorption measurements were performed by using an optical fibre Avantes VIS/NIR AvaSpec-2048 spectrophotometer with light source tungsten-halogen Avalight-HAL and characterised on the CIE 1976 (L, a, b) colour space.

2. Synthesis and characterization

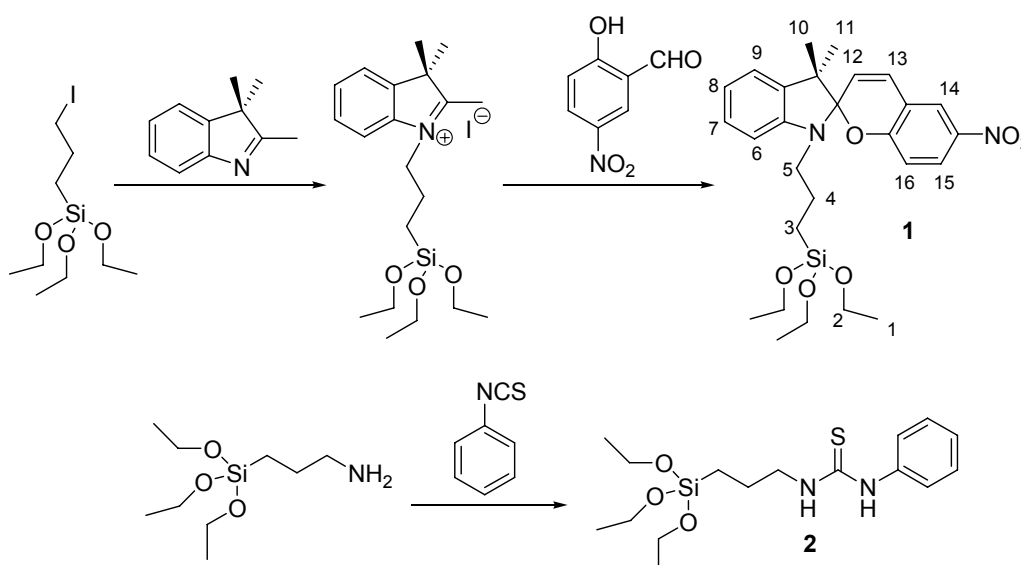
2.1 Synthesis of 1'-(3-triethoxysilanpropyl)-3'-3'-dimethyl-6-nitrospiro[2H-1]benzopyran-2,2'-indoline (1)

The spirobenzopyran derivative 1'-(3-triethoxysilanpropyl)-3'-3'-dimethyl-6-nitrospiro[2H-1]benzopyran-2,2'-indoline (**1**) was synthesised by a two step procedure (see Scheme 1) via reaction of 2,3,3-trimethylindolenine (1.96 mL, 12 mmol) and (3-iodopropyl)triethoxysilane (4.96 mL, 24 mmol) in refluxing ethanol (40mL) for 24h.¹ The product 1-(3-triethoxysilanpropyl)-2,3,3-trimethylindolenine precipitates from ethyl ether (ca. 3 mL). In a further step, reaction of 1-(3-triethoxysilanpropyl)-2,3,3-trimethylindolenine (2.55 g, 7 mmol) with 2-hydroxy-5-nitrobenzaldehyde (1.50 g, 9 mmol) and triethylamine (9 mL) in ethanol (135 mL). The triethoxysilyl derivative **1** was purified in a silica gel column using ethyl acetate-hexane 2:1 as eluent (0.85 g, 17% yield). ^1H -NMR (300 MHz, CDCl_3) δ : 0.55 (t, 2H, H-3), 1.16 (t, 9H, H-1), 1.25 (s, 6H, H-10 and H-11), 1.72 (qn, 2H, H-4), 3.75 (q, 6H, H-2), 3.81 (t, 2H, H-5), 5.84 (d, 1H, H-12), 6.58 (d, 1H, H-9), 6.73 (d, 1H, H-16), 6.84 (t, 1H, H-7), 6.87 (d, 1H, H-13), 7.06 (d, 1H, H-6), 7.14

(t, 1H, H-8), 7.97 (s, 1H, H-14), 8.00 (d, 1H, H-15). ^{13}C -NMR (75 MHz, CDCl_3) δ : 10.5 ($-\text{CH}_2\text{-Si-}$), 20.1 ($(\text{CH}_3\text{-CH}_2\text{-O-Si-})$), 23.5 ($(\text{CH}_3)_2\text{-C}$), 26.2 ($-\text{CH}_2\text{-CH}_2\text{-Si-}$), 27.7 ($(\text{CH}_3)_2\text{-C}$), 44.6 ($(\text{CH}_3)_2\text{-C}$), 46.4 ($\text{N-CH}_2\text{-CH}_2\text{-CH}_2\text{-Si}$), 52.9 ($\text{CH}_3\text{-CH}_2\text{-O-Si-}$), 106.9 ($-\text{N-C-O-}$), 115.8 (C_6H_4), 118.7 (CH=CH), 119.3 (C_6H_3), 119.7 (C_6H_4), 122.2 (C_6H_4), 123.1 (C_6H_3), 126.1 (C_6H_3), 127.9 (C_6H_4), 128.4 (C_6H_3), 130.1 (C_6H_4), 131.9 (CH=CH), 141.1 (C_6H_3), 147.3 (C_6H_4), 159.8 (C_6H_3). Elemental analysis, calculated for $\text{C}_{27}\text{H}_{36}\text{O}_3\text{SiN}_2\text{O}_6$: C, 63.3; H, 7.1; N, 5.5; found: C, 63.1; H, 7.2; N, 5.6.

2.2 Synthesis of *N*-phenyl-*N'*-[3-(triethoxysilyl)propyl]thiourea (2)

N-phenyl-*N'*-[3-(triethoxysilyl)propyl]thiourea (**2**) was synthesized by the reaction between 3-aminopropyltriethoxysilane (4.77 mL, 27 mmol) and phenylisothiocyanate (3.27 mL, 27 mmol) in CH_2Cl_2 (10 mL) and with few drops of triethylamine (see scheme 1). After 24 hours the solvent was removed by evaporation and the product isolated as yellowish oil (7.6 g, 90% yield). ^1H -NMR (300 MHz, CDCl_3) δ : 0.55 (t, 2H, $-\text{CH}_2\text{-Si-}$), 1.60 (t, 2H, $-\text{CH}_2\text{-CH}_2\text{-Si-}$), 3.47 (s, 9H, $\text{CH}_3\text{-O-}$), 3.60 (t, 2H, $-\text{NH-CH}_2\text{-CH}_2\text{-}$), 6.20 (s, 1H, $-\text{CO-NH-CH}_2\text{-}$), 7.12 (m, 5H, $\text{C}_6\text{H}_5\text{-NH-}$), 7.78 (s, 1H, $\text{C}_6\text{H}_5\text{-NH-CO-}$). ^{13}C -NMR (75 MHz, CDCl_3) δ : 6.2 ($-\text{CH}_2\text{-Si-}$), 22.1 ($-\text{CH}_2\text{-CH}_2\text{-Si-}$), 47.8 ($-\text{NH-CH}_2\text{-CH}_2\text{-}$), 50.5 ($\text{CH}_3\text{-O-}$), 125.3 ($\text{C}_6\text{H}_5\text{-NH-}$), 127.2 ($\text{C}_6\text{H}_5\text{-NH-}$), 130.1 ($\text{C}_6\text{H}_5\text{-NH-}$), 136.0 ($\text{C}_6\text{H}_5\text{-NH-}$), 181.0 ($-\text{NH-CO-NH-}$). Elemental analysis, calculated for $\text{C}_{16}\text{H}_{28}\text{O}_3\text{SiN}_2\text{S}$: C, 53.9; H, 7.9; N, 7.9; S 9.0; found: C, 53.8; H, 7.8; N, 8.0; S, 9.1.



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

2.3 Preparation of coated silica nanoparticles

The preparation of the photocromic silica nanoparticles (**N1**) was carried out by the grafting of compounds **1** and **2** by using standard procedures.² In a typical reaction 5 mL of a 30 % suspension of ludox silica nanoparticles AS-30 Colloidal Silica were added to a solution containing 20 mL of acetic acid and 40 mL of ethanol. Then 0.2 mmol of **1** and 1.2 mmol of **2** (dissolved in chloroform) were added to the nanoparticle solution. The crude reaction was heated at 80 °C for 24 hours, the ethanol was evaporated and then the acetic acid solution neutralized with a saturated solution of sodium hydrogencarbonate. The 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 spiropyrane derivative **1** were synthesized (**N2**) as model derivative for the elucidation of the role played by the anionic binding site. 5 mL of a 30 % suspension of ludox silica nanoparticles AS-30 were added to a solution containing 20 mL of acetic acid and 40 mL of ethanol. Then 0.2 mmol of **1** (also dissolved in chloroform) were added to the nanoparticle solution. The crude reaction was heated at 80 °C for 24 hours, the ethanol was evaporated and then the acetic acid solution neutralized with a saturated solution of sodium hydrogencarbonate. The functionalized nanoparticles (**N2**) precipitated and were isolated by filtration, washed with water and acetone and dried at 70 °C. The contents in thiourea and spiropyrane were determined from thermogravimetric, elemental analyses and X-ray absorption microanalyses. The results obtained were reflected in table 1.

Table 1. Typical contents of thiourea and spiropyrane in **N1** and **N2**.

	Content of 1 (mmol/mol SiO ₂)	Content of 2 (mmol/mol SiO ₂)
N1	16	4
N2	17	-

Thermogravimetric analyses were carried out under a flow of air and with a heating rate of 10 °C/minute in the 30-1000 °C interval. The final solid was maintained at 1000 °C for 30 minutes. In the obtained thermograms of **N1** three clearly defined zones are observed; (i) from 30 °C to 150 °C which was assigned to loss of water and organic solvents (2.44 %), (ii) from 150 °C to 800 °C which was assigned to the organic matter attached into the nanoparticle surface (5.97 %) and, finally (iii) from 800° to 1000° C a third step was assigned to condensation of silanol groups (0.44 %). The silica residue amounts to 91.15 %.

The diameter of **N1** and **N2** nanoparticles was determined by TEM. TEM images show a very homogenous particle size around 20 nm (see Figure 1) for both materials.

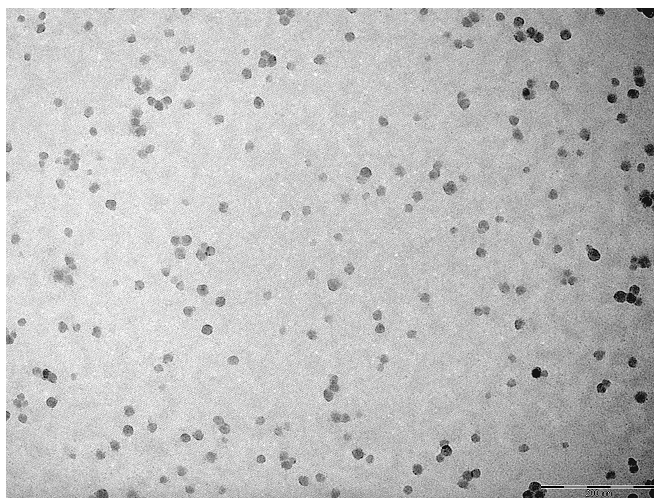


Figure 1. TEM images of functionalized nanoparticles **N1**, average diameter = 20 nm.

3. Carboxylate sensing

The potential sensing behaviour of **N1** nanoparticles in water (HEPES 0.001 mol dm⁻³ at pH 7) was studied against a family of carboxylates of increasing hydrophobicity (acetate, butanoate, hexanoate, octanoate, decanoate and dodecanoate), certain inorganic anions (fluoride, chloride, bromide, iodide, cyanide, sulfate, phosphate and nitrate) and lauryl sulfate. In a typical experiment, 20 mg of **N1** were added to a 2 ml solution of the respective anion ($C_{anion} = 1 \times 10^{-3}$ mol dm⁻³) in HEPES (1 x 10⁻³ mol dm⁻³ at pH 7) and were suspended with a rotor-stator system (Ultra-Turrax) for 4 minutes and ultrasonicated at 25 °C. Then, and in order to quantify the colour modulation upon addition of different anions, the colour of the water-**N1** suspensions were measured by using an optical fibre UV-visible spectrophotometer and characterised on the CIE 1976 (L, a, b) colour space.³ This system is one of the most complete colour mode conventionally used to describe visible colours to the human eye. It consist 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 systems each colour is a point in a three dimensional space and the relative difference between two any colours is given by ΔE that represents the Euclidean distance between them.

$$\Delta E_{ab} = \sqrt{(L_2 - L_1)^2 + (a_2 - a_1)^2 + (b_2 - b_1)^2}$$

Of all the anions tested only carboxylates are able to induced changes in the colour of **N1** nanoparticles (from magenta/red to light pink) whereas the addition of inorganic anions and lauryl sulfate induced negligible changes in the colour of **N1** nanoparticle suspension. The coordinates L, a and b and the difference between colours as ΔE values of **N1** and **N1** nanoparticles upon addition of the respective carboxylate are listed in table 2. As could 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 self assembled monolayer of different degrees of hydrophobicity around the signalling reporter.

Table 2. Quantify of colour modulation upon addition of different carboxilates characterised on the CIE 1976 (L, a, b) colour space. The coordinates represent the lightness of the colour (L) and its position between magenta/green and yellow/blue (a and b respectively). The relative difference between two any colours is given by ΔE .

	L	a	b	ΔE
No carboxylate	77,590	37,77	-0,340	
Acetate	79,780	31,80	3,020	7,196
Butanoate	81,650	29,45	5,060	10,721
Hexanoate	87,575	26,76	6,820	16,498
Octanoate	90,483	23,84	4,997	19,717
Decanoate	90,430	22,09	5,980	21,229
Dodecanoate	93,457	21,43	8,925	24,587

The gradual colour change from magenta/red to light pink upon carboxylate coordination is clearly linked with the length of the alkyl chain. The polarity-controlled mechanism was assessed by the fact that the addition of the inorganic anions (fluoride, chloride, bromide, iodide, cyanide, sulfate, phosphate and nitrate) and lauryl sulfate induced negligible colour changes in **N1** aqueous suspension.

The same carboxyalate sensing experiences were carried out with aqueous suspensions of **N2** nanoparticles (i.e. functionalised with the signalling reporter but no containing thiourea groups). Again 20 mg of **N2** were added to a 2 ml solution of the respective anion ($C_{anion} = 1 \times 10^{-3} \text{ mol dm}^{-3}$) in HEPES ($1 \times 10^{-3} \text{ mol dm}^{-3}$ at pH 7) and were suspended with a rotor-stator system (Ultra-Turrax) for 4 minutes and ultrasonicated at 25 °C. Then, the colour of the water-**N2** suspensions were measured by using an optical fibre UV-visible spectrophotometer and characterised again on

the CIE 1976 colour space. These nanoparticle suspensions do not show any noticeable colour change (reflected in the L, a, b values) in the presence of all the anions tested (acetate, butanoate, hexanoate, octanoate, decanoate, dodecanoate, fluoride, chloride, bromide, iodide, cyanide, sulfate, phosphate, nitrate and lauryl sulfate) pointing out the relevance that the presence of the thiourea moieties has in relation to the development of the colour modulation and suggesting that this colour modulation is not due to carboxylate interaction with the nanoparticle surface but most likely due to polarity-induced effects.

Titration experiences with **N1** nanoparticles and hexanoate and dodecanoate were carried out. In these experiences several UV-visible measurements of **N1** nanoparticles with different amounts of hexanoate and dodecanoate (0, 0.3, 0.5, 0.7, 1.0, 2.0 and 3.0 equivalents related to the content in thiourea moiety in the nanoparticles) were performed. In a typical experiment, 20 mg of **N1** were added to solutions of hexanoate and dodecanoate ($C_{anion} = 3 \times 10^{-3} \text{ mol dm}^{-3}$) in HEPES ($1 \times 10^{-3} \text{ mol dm}^{-3}$ at pH 7) and were suspended with a rotor-stator system (Ultra-Turrax) for 4 minutes and ultrasonicated at 25 °C. Then, and in order to quantify the colour modulation upon addition of different quantities of hexanoate and dodecanoate, the colour of the water-**N1** suspensions were measured by using an optical fibre UV-visible spectrophotometer and characterised on the CIE 1976 (L, a, b) colour space. The titration curves obtained are shown in Figure 2.

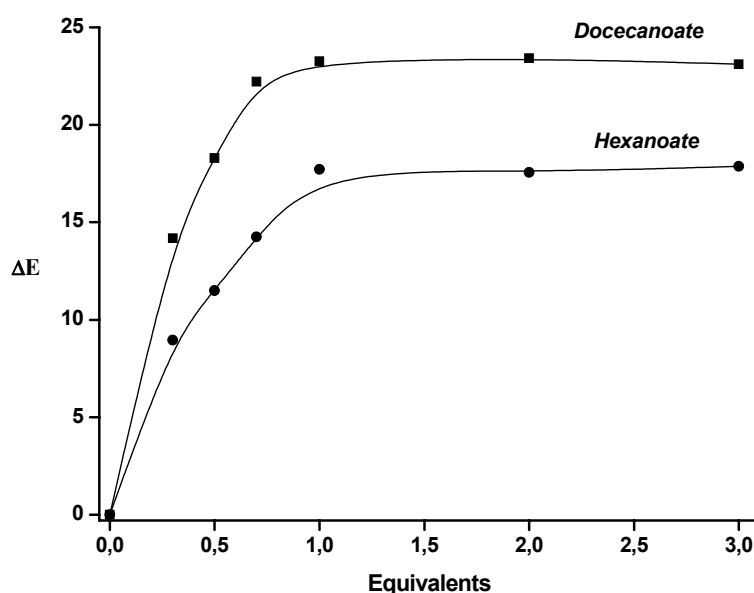


Figure 2. Titration curves of functionalized nanoparticles **N1** obtained upon addition of increasing quantities of hexanoate and dodecanoate.

Also the colorimetric response of water-**N1** suspensions in the presence of certain inorganic anions (fluoride, chloride, bromide, iodide, cyanide, sulfate, phosphate and nitrate) has been

studied. The sensing procedure is the same described above for the sensing of linear alkyl carboxylates. In a typical experiment, 20 mg of **N1** were added to a 2 ml solution of the respective inorganic anion ($C_{anion} = 1 \times 10^{-3} \text{ mol dm}^{-3}$) in HEPES ($1 \times 10^{-3} \text{ mol dm}^{-3}$ at pH 7) and were suspended with a rotor-stator system (Ultra-Turrax) for 4 minutes and ultrasonicated at 25 °C. Then, and in order to quantify the colour modulation upon addition of different anions, the colour of the water-**N1** suspensions were measured by using an optical fibre UV-visible spectrophotometer and characterised on the CIE 1976 (L, a, b) colour space. The presence of this inorganic anions induced negligible changes neither in the colour nor in the CIE Lab coordinates in water-**N1** suspension. This fact points directly to a polarity-controlled mechanism as a responsible of the colour changes observed upon addition of certain linear alkyl carboxylates.

4. References

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