Direct synthesis and fluorescent imaging of bifunctionalized mesoporous iodopropyl-silica

Ramm, J H; Gartmann, N; Brühwiler, D
Direct synthesis and fluorescent imaging of bifunctionalized mesoporous iodopropyl-silica

Jan H. Ramm, Nando Gartmann, Dominik Brühwiler*

Institute of Inorganic Chemistry, University of Zurich, Winterthurerstrasse 190,
CH-8057 Zurich, Switzerland

* Corresponding author. Tel.: +41 44 635 4630; fax: +41 44 635 6802. E-mail address: bruehwi@aci.uzh.ch
Abstract

The co-condensation of 3-iodopropyltrimethoxysilane and tetraethoxysilane with an additional substituted trimethoxysilane (RTMS) in the presence of Pluronic P123 and hydrogen iodide yields bifunctionalized mesoporous silica. The pore size distribution of these materials depends on the nature of the RTMS additive. Excellent results in terms of a narrow pore size distribution were obtained with methyltrimethoxysilane. A particularly interesting bifunctionalized mesoporous material is formed by the co-inclusion of iodopropyl and aminopropyl moieties. The complementary reactivity of these two functional groups is demonstrated by the selective labeling of the amino-iodo-functionalized mesoporous silica with 2-hydroxy substituted nile red and fluorescein isothiocyanate, allowing further characterization of the functional group distribution by confocal laser scanning microscopy.

Keywords: mesoporous silica, co-condensation, iodopropyl, aminopropyl, labeling, confocal laser scanning microscopy.
1. Introduction

Bifunctionalized mesoporous silica has recently attracted increased interest in the fields of catalysis [1], adsorption [2], and sensing [3]. A variety of functional group pairs have been explored, including sulfonic acid/amino [1d], sulfonic acid/mercapto [1e], amino/carboxyl [4], mercapto/acetylacetonato [5], and amino/phenyl [6]. Among the many functional groups that have been incorporated into mesoporous silica, the iodopropyl group stands out as a particularly interesting option in terms of the intriguing possibilities for further modification by means of nucleophilic substitution. Alauzun et al. have shown that the direct synthesis of iodopropyl-functionalized SBA-15 type materials is successful if HCl is replaced by HI to avoid the formation of chloropropyl groups [7]. Based on this procedure, we have investigated the direct synthesis of bifunctionalized mesoporous silica containing iodopropyl as primary functional group. Additional organic groups can for example be envisaged to provide pores with increased hydrophobicity, which for many applications, including catalysis, sensing, low $k$ dielectrics, and drug delivery, might be more desirable than the environment created by the abundant surface hydroxyl groups common to mesoporous silica based materials after removal of the respective structure-directing agent (SDA) [8]. On the other hand, the presence of surface groups with complementary reactivity, such as iodopropyl and aminopropyl, is expected to facilitate further modification towards multifunctionalized materials.

The inclusion of functional groups into mesoporous silica is most conveniently accomplished by a co-condensation approach (also referred to as direct synthesis or one-pot synthesis), as postsynthetic modification often leads to a preferential functionalization of the most accessible sites (external surface and pore entrances)
[9,10]. However, the quality of the porous inorganic-organic hybrid materials resulting from co-condensation depends on the nature of the respective alkoxy silane precursors. Having an organic group that is sufficiently hydrophobic to interact with the core of the micelles is often advantageous.

Obtaining information on the distribution of the functional groups is a key issue in the synthesis of functionalized mesoporous silicas. Confocal laser scanning microscopy (CLSM) offers intriguing possibilities in this regard, and has already been successfully applied to zeolites [11] and mesoporous silicas [12] containing luminescent guests, as well as for determining the distribution of aminopropyl moieties after postsynthetic modification [9]. In order to apply CLSM to bifunctionalized mesoporous silica, 3-iodopropyltrimethoxysilane (IPTMS) was co-condensed with 3-aminopropyltrimethoxysilane (APTMS). This results in a material containing functional groups that can be labeled independently with fluorescent moieties.

2. Materials and methods

2.1. Synthesis of bifunctionalized mesoporous iodopropyl-silica

The preparation of mesoporous silica containing iodopropyl groups and additional organic moieties is derived from the synthesis of iodopropyl-functionalized mesoporous silica reported by Alauzun et al. [7]. Briefly, an amount of 2.00 g of Pluronic P123 (EO\textsubscript{20}PO\textsubscript{70}EO\textsubscript{20}, M\textsubscript{av} = 5800, Aldrich) was dissolved in 80 mL of H\textsubscript{2}O containing 0.58 mL of aqueous HI (55–58 %, Fluka). This solution was added to a mixture containing 4.75 mL (21 mmol) of tetraethoxysilane (TEOS, Fluka, ≥ 99 %), 29.6 µL (0.15 mmol) or 220 µL (1.12 mmol) of 3-iodopropyltrimethoxysilane (IPTMS, ABCR Karlsruhe), and a specific amount of an additional substituted trimethoxysilane RTMS (Table 1).
The mixture was stirred at room temperature for 90 min. After heating to 60 °C, 0.04 g of NaF was added, and the mixture was stirred for 72 h at 60 °C. The product was recovered by filtration. After washing with 200 mL of acetone, the SDA was removed by Soxhlet extraction with ethanol over a period of 24 h. The resulting white solid was oven-dried at 60 °C, yielding typically around 1.4 g of product. Particles with irregular morphology and sizes ranging from 2 µm to larger aggregates of ca. 50 µm were obtained.

Methyltrimethoxysilane (Aldrich, ≥ 98 %), phenyltrimethoxysilane (Aldrich, 97 %), octyltrimethoxysilane (Aldrich, 96 %), octadecyltrimethoxysilane (Aldrich, ≥ 90 %), 3-aminopropyltrimethoxysilane (Fluka, ≥ 97 %), and isobutyltrimethoxysilane (ABCR Karlsruhe, 97 %) were used as received.

2.2. Synthesis and coupling of 2-hydroxy substituted nile red

2.2.1. 5-Diethylamino-2-nitrosophenol (DNP)

An amount of 3.36 g (20 mmol) of 3-diethylaminophenol (Aldrich, 97 %) was dissolved in 8 mL of 32 % aqueous HCl and 20 g of ice. A solution of 1.68 g (24 mmol) of NaNO₂ in 6 mL of water was added dropwise (during 1 h) to the above solution and the temperature was kept between 0 and 5 °C. The resulting brown slurry was stirred for 2 h. Following filtration and washing with 10 mL of 4 M aqueous HCl, the product was dried under vacuum and recrystallized with 150 mL of ethanol. After addition of 20 mL of diethylether, the suspension was cooled to 0 °C and kept over night for crystallization. The precipitate was filtered, washed with a few milliliters of ethanol and dried under vacuum yielding 3.33 g (14 mmol, 71 %) of a yellow product (DNP hydrochloride). The DNP hydrochloride can be converted to the free compound by
diluting in 100 mL of 0.1 M aqueous NaHCO₃, followed by an extraction with 100 mL and two times 50 mL of dichloromethane, with subsequent drying of the organic phase over Na₂SO₄. ¹³C NMR (400 MHz, [²H₆]DMSO): δ = 169.6, 158.0, 150.1, 135.4 (CH), 116.3 (CH), 96.0 (CH), 46.5 (NCH₂CH₃), 14.9 (NCH₂CH₃). ¹H NMR (400 MHz, [²H₆]DMSO): δ = 7.32 (1H, d, J = 10 Hz), 6.90–6.87 (1H, m), 5.74 (1H, d, J = 3 Hz), 3.60–3.58 (4H, m), 1.20 (6H, t, J = 7.2 Hz). IR (KBr)/cm⁻¹: 2980, 2937, 1629, 1514.

2.2.2. 9-Diethylamino-2-hydroxy-5H-benzo[a]phenoxazin-5-one (NR–OH)

2-Hydroxy substituted nile red (NR–OH), which we use as a fluorescent label for the co-condensed iodopropyl groups, was synthesized according to reference 13. Briefly, DNP hydrochloride (1.28 g, 5.5 mmol) and 1,6-dihydroxynaphthalene (0.90 g, 5.6 mmol, ABCR Karlsruhe, 97 %) were dissolved in dry DMF and heated for 4 h under reflux. The solvent was removed under reduced pressure to yield a dark blue-green solid. Subsequent purification by column chromatography (ethyl acetate/isopropanol 4:1) resulted in 0.66 g (2.0 mmol, 36 %) of a dark brown solid. ¹³C NMR (500 MHz, [²H₆]DMSO): δ = 182.5 (CO), 161.5, 152.5, 151.6, 147.3, 139.6, 134.6, 131.7 (CH), 128.3 (CH), 124.8, 119.3 (CH), 110.8 (CH), 109.0 (CH), 105.0 (CH), 97.0 (CH), 45.3 (NCH₂CH₃), 13.3 (NCH₂CH₃). ¹H NMR (500 MHz, [²H₆]DMSO): δ = 10.41 (1H, s), 7.98 (1H, d, J = 8.5 Hz), 7.89 (1H, d, J = 2 Hz), 7.59 (1H, d, J = 9.5 Hz), 7.10 (1H, dd, J = 8.5 Hz and 2.5 Hz), 6.82 (1H, dd, J = 9 Hz and 2.5 Hz), 6.64 (1H, s, J = 2.5 Hz), 6.15 (1H, s), 3.52 (4H, q, J = 7 Hz ), 1.18 (6H, t, J = 7 Hz).
2.2.3. Coupling of NR–OH to iodopropyl groups

Functionalized mesoporous silica (250 mg) was dispersed in 5 mL of dry DMF and 20 mg (60 µmol) of NR–OH was added. After the addition of 45 mg of K₂CO₃, the mixture was stirred under nitrogen for 24 h at room temperature. The product was recovered by centrifugation and washed with 100 mL of 0.1 M aqueous HCl. The silica was subsequently dispersed in 20 mL of water for 10 min and recovered by centrifugation. This step was repeated until the washing solution had a pH above 6 (typically requiring three washing steps). After a final washing with 100 mL of ethanol, the samples were Soxhlet extracted with ethanol and dried at 60 °C. The amount of coupled NR–OH was determined by dissolving 10–20 mg of the dry sample in 20 mL of 0.1 M NaOH (in ethanol/water 1:1) and measuring the UV-Vis absorption spectrum of the resulting solution (εₑₓₘₐₓ = 22000 M⁻¹cm⁻¹).

2.3. Coupling of fluorescein isothiocyanate

Labeling of the amino groups in iodopropyl-aminopropyl-functionalized mesoporous silica was accomplished as follows: A calculated amount of fluorescein isothiocyanate (FITC, Fluka, isomer I, ≥ 97.5 %, 1.5-fold excess relative to the amount of amino groups) was dissolved in 25 mL of absolute ethanol. After the addition of 80 mg of functionalized mesoporous silica, the suspension was stirred for 24 h at room temperature. The labeled product was recovered by filtration, washed with ethanol, and dried at 80 °C. The amount of coupled FITC was determined by dissolving 15–30 mg of the sample in 25 mL of 0.2 M aqueous NaOH and measuring the UV-Vis absorption spectrum of the resulting clear solution (εₑₓₘₐₓ = 75000 M⁻¹cm⁻¹) [14].
2.4. Characterization

Nitrogen sorption isotherms were collected at 77 K with a Quantachrome NOVA 2200. Samples were vacuum-degassed at 80 °C for 3 h. Mesopore size distributions were evaluated from the adsorption branches of the nitrogen isotherms by means of the BJH method [15]. The total surface area $S_{BET}$ was calculated by the BET method [16]. The total pore volume $V_{tot}$ was determined from the amount of nitrogen adsorbed at a relative pressure of 0.98. Photoluminescence spectra were recorded with a Perkin-Elmer LS50B spectrofluorometer equipped with a front surface accessory for the measurement of powdered samples. UV-Vis absorption spectra were measured in ethyl benzoate as an index-matching solvent ($n = 1.504$). Elemental (CHN) analysis of the mesoporous materials was performed with a LECO CHNS-932. The iodine content of the samples was analyzed following a Schöniger digestion [17]. Scanning electron microscopy images were acquired on a JEOL JSM-6060. The confocal laser scanning microscopy setup consisted of an Olympus BX 60 microscope equipped with a FluoView confocal unit and lasers operating at 488 (Ar ion) and 543.5 nm (He/Ne).

3. Results and discussion

3.1. General observations

The nitrogen sorption isotherms of samples synthesized with an IPTMS/RTMS ratio of 1:10 are shown in Figure 1. The corresponding pore size distributions (PSDs) are given in Figure 2 with additional characterization data compiled in Table 1. Comparison with the monofunctionalized sample (Ip) reveals that the addition of methyltrimethoxysilane (MTMS) to the synthesis mixture does not cause a significant change in the porosity of the material (Ip/Me). The course of the respective adsorption
isotherm at a relative pressure above 0.80 even suggests that Ip/Me features less textural porosity. This observation is supported by the pore volumes calculated at relative pressures of 0.98 and 0.80. In the case of Ip/Me, the total pore volume $V_{\text{tot}}$ ($p/p_0 = 0.98$) is 1.27 cm$^3$/g, whereas $V_{0.80}$ ($p/p_0 = 0.80$) amounts to 1.02 cm$^3$/g. For Ip, the difference between these two values is significantly larger ($V_{\text{tot}} = 1.65$ cm$^3$/g, $V_{0.80} = 0.86$ cm$^3$/g).

The presence of the methyl groups in Ip/Me furthermore leads to a decrease of the $C_{\text{BET}}$ value (Table 1). The $C_{\text{BET}}$ parameter is related to the energy of interaction of nitrogen with the surface and is therefore a rough estimate for the polarity. $C_{\text{BET}}$ values close to 100 are characteristic of hydroxylated silica surfaces, whereas modification with hydrophobic groups generally reduces $C_{\text{BET}}$ [18].

![Figure 1. Nitrogen adsorption (●) and desorption isotherms (○) of mesoporous iodopropyl-silica (Ip) and of bifunctionalized mesoporous iodopropyl-silica.](image)
Figure 2. Pore size distributions of mesoporous iodopropyl-silica (Ip) and of bifunctionalized mesoporous iodopropyl-silica calculated from the adsorption isotherms shown in Figure 1.

Compared to Ip and Ip/Me, co-condensation of IPTMS with isobutyltrimethoxysilane or phenyltrimethoxysilane produced samples with smaller pore diameter (Ip/iBu and Ip/Ph) and, particularly in the case of Ip/Ph, a less defined PSD. Interestingly, the sample synthesized with octyltrimethoxysilane (Ip/Oc) does not possess defined mesopores, whereas the sample prepared with octadecyltrimethoxysilane (Ip/Od) features a comparatively well-defined PSD with a maximum at 9.1 nm. It seems that the interaction of the SDA with octadecyl chains is more favorable for the formation of a defined pore structure than the interaction with the shorter octyl chains. Irrespective of the employed RTMS, C<sub>BET</sub> values are lower than for the sample synthesized in the absence of a RTMS additive. This is in agreement with the presence of hydrophobic groups on the pore surface of the bifunctionalized samples.

The determination of the absolute amount of incorporated R moieties is complicated by a slight esterification of the silica surface upon extraction with ethanol [19] and the potential presence of residual SDA. The values given in Table 1 were
therefore obtained by taking Ip as a reference. In accordance with the relative amounts of IPTMS and RTMS in the synthesis mixture, the n(R)/n(I) ratios in the final products are reasonably close to 10. More importantly, we can conclude that the presence of a RTMS additive does not cause a significant change of the amount of incorporated iodopropyl moieties. In all cases, scanning electron microscopy images showed particles with irregular morphology and sizes ranging from 2 µm to larger aggregates of ca. 50 µm.

3.2. High iodopropyl content

In terms of the PSD, the best results were obtained with MTMS as an additive (Figure 2). We have therefore conducted the same synthesis with an increased amount of IPTMS (samples Ip-1.1 and Ip-1.1/Me). The 7.5-fold increase of the IPTMS concentration led to a substantially smaller pore size (Figure 3). Interestingly, the pore size difference between Ip-1.1 and Ip is retained after removal of the grafted iodopropyl moieties by calcination (550 °C, 16 h, Figure 4), thus indicating that the amount of IPTMS in the reaction mixture has a significant effect on the condensation of the silica framework.
Figure 3. Pore size distributions of Ip-1.1 (1.12 mmol of IPTMS, □) and Ip-1.1/Me (1.12 mmol of IPTMS and 1.5 mmol of MTMS, ×). The corresponding nitrogen sorption isotherms are shown in the inset.

Figure 4. Pore size distribution of Ip-1.1 (○) and Ip (●) after removal of the iodopropyl moieties by calcination.

The bifunctionalized sample Ip-1.1/Me is superior to the monofunctionalized sample Ip-1.1 in terms of containing a minimum relative amount of textural porosity. This is in agreement with the results obtained from the corresponding samples synthesized with a smaller concentration of IPTMS (Ip and Ip/Me). Furthermore, the
addition of MTMS obviously does not affect the incorporation of IPTMS into the silica framework, as indicated by the iodo contents of Ip-1.1 and Ip-1.1/Me (Table 1).

3.3. Fluorescent labeling and confocal laser scanning microscopy

The use of APTMS as an additional precursor offers possibilities for subsequent fluorescent labeling and for the analysis of the functional group distribution by CLSM. A narrow PSD is obtained when using 0.15 mmol of each IPTMS and APTMS (Figure 5). Similar to the materials synthesized with alkyltrimethoxysilanes, the iodo- and aminopropyl containing sample (Ip/Ap) features significant textural porosity, as evident from the hysteresis in the high pressure section of the nitrogen sorption isotherm (Figure 5, inset).

Figure 5. Pore size distribution of Ip/Ap. The corresponding nitrogen sorption isotherms are shown in the inset together with a scanning electron microscopy image of a Ip/Ap particle. The length of the scale bar is 10 µm.

The co-condensed iodopropyl and aminopropyl groups can be labeled selectively with NR–OH and FITC, respectively (Figure 6). No labeling was observed upon
reaction of NR–OH with mesoporous silica containing only aminopropyl groups. FITC, on the other hand, did not bind to iodopropyl-silica in the absence of aminopropyl groups. However, the consecutive reaction of Ip/Ap with NR–OH and FITC yields a material containing both fluorescent labels. Analysis by UV-Vis spectroscopy revealed a nile red content of 5 µmol/g and a fluorescein content of 14 µmol/g. The absorption spectrum of the powdered sample measured in an index-matching solvent (ethyl benzoate, Figure 7, bottom panel) features three maxima at 462, 488, and 537 nm, as well as a shoulder around 430 nm, corresponding well to the maxima found in the spectra of the materials containing only one of the labels (Figure 7, top panel). The occurrence of two maxima (and a short wavelength shoulder) in the absorption spectrum of coupled FITC indicates the presence of fluorescein moieties in different protonation states (dianion, monoanion, neutral) [20]. The luminescence spectrum of the FITC/NR–OH labeled sample (Figure 7, bottom panel) shows a comparatively weak luminescence of the coupled FITC. This can be attributed to the considerable overlap between the emission band of the FITC labels and the absorption band of the NR–OH labels, leading to radiative energy transfer. Assuming a homogeneous distribution of the fluorescent labels on the surface of Ip/Ap, a density of roughly 0.016 nm\(^{-2}\) is obtained, corresponding to one label per 60 nm\(^2\). Additionally taking into account the fact that most of the surface of the material is concave, leading for example to situations where labels occupy sites on opposing channel walls, non-radiative energy transfer might contribute to the low luminescence intensity of the coupled FITC labels. Nonetheless, the intensity of the fluorescein emission is still sufficient for imaging by CLSM.
Figure 6. Labeling of iodopropyl-aminopropyl-functionalized mesoporous silica with 2-hydroxy substituted nile red (NR–OH) and fluorescein isothiocyanate (FITC).
Figure 7. Absorption and luminescence spectra of Ip/Ap after either FITC labeling (dashed curves, top panel, excitation at 450 nm) or NR–OH labeling (solid curves, top panel, excitation at 490 nm). The bottom panel shows the absorption and luminescence spectra (excitation at 450 nm) of an Ip/Ap sample after labeling with NR–OH and FITC as schematically shown in Figure 6.

CLSM allows us to selectively image the fluorescein and nile red emission of the particles. A non-uniform spatial distribution of the luminescence intensity is usually a sign of a non-uniform distribution or insufficient accessibility of the incorporated functional groups [9,21]. The bifunctionalized mesoporous iodopropyl-silica particles are relatively large and feature irregular morphology. Observing optical slices at the center of such particles therefore provides representative information on the distribution of the fluorescent moieties. The spatial distribution of the coupled FITC (green) and
coupled NR–OH (red) is very similar (Figure 8). This indicates that the distribution of the amino- and iodopropyl groups in Ip/Ap is uniform, as is commonly expected for co-condensed mesoporous silica samples. Furthermore, and maybe more importantly, amino- and iodopropyl groups located in the center of the particles are accessible. It is very likely that the relatively pronounced textural porosity of the samples contributes to the accessibility of the functional groups.

Figure 8. Confocal laser scanning microscopy images of two Ip/Ap particles after labeling with NR–OH and FITC. Excitation was performed at 488 and 543.5 nm. Optical slices in the center of the particles were selected. The left (green) images show the luminescence of the coupled FITC labels, whereas the right (red) images display the luminescence of coupled NR–OH.

4. Conclusions

IPTMS and RTMS can be co-condensed with TEOS in the presence of Pluronic P123 as a structure-directing agent to yield bifunctionalized mesoporous silica. The best results in terms of a narrow pore size distribution were obtained with R = methyl. As evident from the low \( C_{\text{BET}} \) values, the inclusion of alkyl moieties causes a change in surface properties, thus opening possibilities to tailor the environment of the iodopropyl
sites. The co-inclusion of iodopropyl and aminopropyl groups is particularly interesting due to their complementary reactivity. Mesoporous silica providing iodo and amino groups as anchoring sites can serve as a starting material for the synthesis of mesoporous inorganic-organic hybrid materials with multiple functionality. Selective fluorescent labeling enables the imaging of the spatial distribution of the incorporated amino and iodo groups by confocal laser scanning microscopy. The results indicate that the organic moieties in bifunctionalized mesoporous iodopropyl-silica are uniformly distributed and that the porosity renders groups located in the center of the particles accessible.

**Acknowledgments.** Financial support was provided by the European Commission through the Human Potential Program (Marie-Curie RTN Nanomatch, Grant No. MRTN-CT-2006-035884) and by the Swiss National Science Foundation (Project 200020-117591).
References


Figure Captions

Figure 1. Nitrogen adsorption (●) and desorption isotherms (○) of mesoporous iodopropyl-silica (Ip) and of bifunctionalized mesoporous iodopropyl-silica.

Figure 2. Pore size distributions of mesoporous iodopropyl-silica (Ip) and of bifunctionalized mesoporous iodopropyl-silica calculated from the adsorption isotherms shown in Figure 1.

Figure 3. Pore size distributions of Ip-1.1 (1.12 mmol of IPTMS, □) and Ip-1.1/Me (1.12 mmol of IPTMS and 1.5 mmol of MTMS, ×). The corresponding nitrogen sorption isotherms are shown in the inset.

Figure 4. Pore size distribution of Ip-1.1 (○) and Ip (●) after removal of the iodopropyl moieties by calcination.

Figure 5. Pore size distribution of Ip/Ap. The corresponding nitrogen sorption isotherms are shown in the inset together with a scanning electron microscopy image of a Ip/Ap particle. The length of the scale bar is 10 µm.

Figure 6. Labeling of iodopropyl-aminopropyl-functionalized mesoporous silica with 2-hydroxy substituted nile red (NR–OH) and fluorescein isothiocyanate (FITC).
Figure 7. Absorption and luminescence spectra of Ip/Ap after either FITC labeling (dashed curves, top panel, excitation at 450 nm) or NR–OH labeling (solid curves, top panel, excitation at 490 nm). The bottom panel shows the absorption and luminescence spectra (excitation at 450 nm) of an Ip/Ap sample after labeling with NR–OH and FITC as schematically shown in Figure 6.

Figure 8. Confocal laser scanning microscopy images of two Ip/Ap particles after labeling with NR–OH and FITC. Excitation was performed at 488 and 543.5 nm. Optical slices in the center of the particles were selected. The left (green) images show the luminescence of the coupled FITC labels, whereas the right (red) images display the luminescence of coupled NR–OH.