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Organically modified xerogels as supports for solid-phase chemistry

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Abstract—Organically modified xerogels (OMX_{NH2}) can be used as an easy to handle and chemically stable support in solid-phase chemistry and are compatible with enzymatic transformations.

The use of silica-based materials and in particular of xero- and aerogels is on the increase in various research areas including materials, analytical and the life sciences.[1] Particularly interesting are the recent applications in biosensors.[2] Aero- and xerogels are prepared by 'sol–gel' processing.[1b] The difference between the two types of materials lies in the method of drying after sol–gel processing: either using supercritical fluids or air. Drying by supercritical fluids leaves highly porous aerogels, while air drying results in shrinking of the material into small particles with only partial retention of the porosity introduced in the sol–gel process. Potential applications of these materials have greatly increased with the introduction of organically modified monomers at the sol–gel processing stage,[3] resulting in organically modified xerogels (OMX). Among the variety of functionalities that can be introduced are amines, thiols, oxiranes, carbamates, and isocyanates and the resulting OMX therefore have promising properties for new types of sensors, catalysts and adsorbants.[3b]

Although silicates have been used before as solid supports for chemical modifications, this is the first report of the use of organically modified xerogels in solid-phase chemistry.[4]

Thanks to their high porosity, good loading, chemical stability and compatibility with aqueous and organic solvents and with many conditions typically used in solid-phase synthesis, these materials could be a cheap alternative to conventional beads used in solid-phase chemistry. In this communication, we show for the first time the successful application of OMX_{NH2} as a support for chemical peptide synthesis followed by enzymatic hydrolysis on solid support.

OMX_{NH2} was prepared by sol–gel processing of a mixture of tetraethoxysilane $Si(OEt)_4$, (TEOS) and (3-aminopropyl) trimethoxysilane $NH_2(CH_2)_3Si(OMe)_3$,[5] (Scheme 1) followed by air drying of the formed wet gels causing the gel to shrink.[†] After drying, the physical appearance of the OMX_{NH2} was a heterogeneous mixture of fragments, with a size distribution of 20–2000 μm (Fig. 1a).

In order to obtain a suitable homogeneous preparation for applications in solid-phase chemistry, the 100–250 μm (OMX_{NH2}-100) and 250–500 μm (OMX_{NH2}-250, see Fig. 1b) fractions were selected. The resulting material was then characterized as follows. First, the total surface area of OMX_{NH2} was determined by BET. The average surface area of the OMX_{NH2}, obtained after air

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[†] Preparation of OMX_{NH2}: 13.3 mL TEOS (12.42 g), 1.34 mL (3-aminopropyl)trimethoxysilane (1.38 g), 27.74 mL MeOH and 5.40 mL H_2O. The gel was dried at room temperature to constant weight. After chemical modifications the solvent content was determined by drying samples at 100°C. The size selection of particles was performed by using a pile of sieves shaken mechanically.

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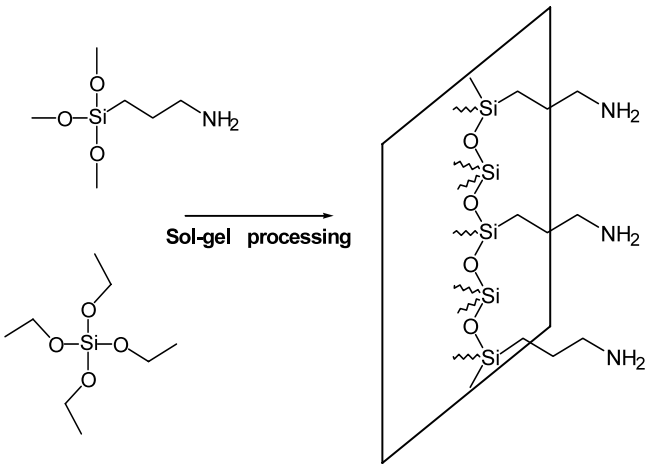


Scheme 1. Sol–gel processing and OMX_{NH2} formation.

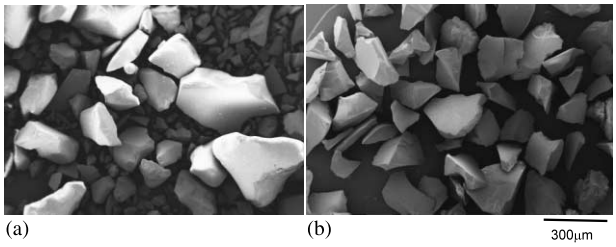
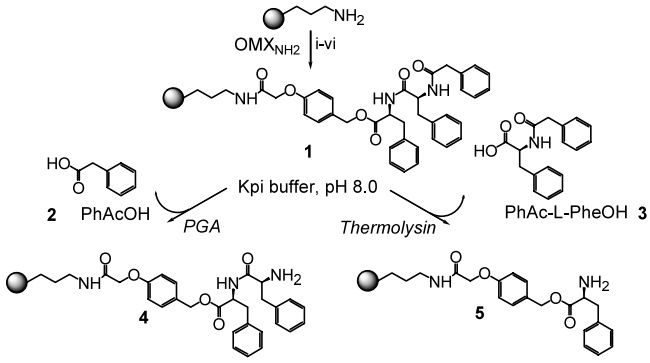


Figure 1. Electron micrograph of the OMX_{NH2} (a) after the air drying process and (b) after the size selection (OMX_{NH2}-250).

drying was 165 m^2/g, comparable to that reported for aerogels obtained starting from the same alkoxides but dried using supercritical fluids (184 m^2/g).[3b] Due to their high surface area, xerogels are likely to be accessible to chemical reactants and thus useful for solid-phase applications. The accessible free amino groups on the solid support were quantified by acid–base titration at a 300 $\mu mol/g_{dry\ support}$.[‡] The amino groups accessible for chemical modification were then determined by exploiting Fmoc chemistry.[§] The chemically accessible amino groups were found to be 250 $\mu mol/g_{dry\ support}$ after three consecutive coupling steps (180 and 220 $\mu mol/g_{dry\ support}$ after the first and second coupling respectively) thus meaning that more than 80% of the available amino groups were chemically accessible to bulkier substrates. The remaining 50 μmol were probably buried in small pores that were accessible to protons in the titration experiment but inaccessible to activated Fmoc-amino acid.

Next, the suitability of the support for a multistep chemical modification was assessed. On both size fractions, dipeptide **1** (Scheme 2) was synthesized while anchored onto the OMX_{NH2} amine via a Wang type linker (4-hydroxymethylphenoxyacetic acid). The synthesis was performed using conventional peptide coupling procedures. During the release of the Fmoc protecting group (step v, Scheme 2), 250 $\mu mol/g_{dry\ support}$ (150 $\mu mol/g_{wet\ support}$) were acylated (Table 1),



Scheme 2. Chemical procedure for the synthesis of OMX_{NH2}-Wang-L-Phe-L-Phe-PhAc **1** and enzymatic catalysed hydrolysis. *Reagents and conditions*: (i) wash with MeOH, DCM, DMF; (ii) HMPA (3 equiv.), DIC/HOBT, DMF, (iii) Fmoc-L-PheOH (3 equiv.), DIC/DMAP, DMF, (iv) acetic anhydride (10 equiv.), DMF; (v) Fmoc deprotection (20% piperidine), DMF; (vi) PhAc-L-PheOH (10 equiv.), HOBT/HBTU/DIPEA, DMF.

Table 1. Yields obtained in the chemical synthesis of **1** and enzymatic hydrolysis by PGA or thermolysin on OMX_{NH2} and $PEGA_{1900}$[a]

Product	OMX_{NH2}		$PEGA_{1900}$
($\mu mol/g_{dry}$)	100–250 μm	250–500 μm	
Dipeptide **1**	250	250	150
PhAcOH **2**	10	12	16
PhAc-L-PheOH **3**	40	45	130

[a] *Reaction conditions*: about 20–60 mg of dry support, 6 mL phosphate buffer (0.1 M, pH 8.0), rt, 5 mg enzyme. The mixture in a reacti-syringe was allowed to mix in a blood rotator 24 h, 40 rpm. The solution was filtered and the support washed with H_2O/MeCN (50/50), the total solution dried and re-dissolved in 1 mL of MeCN/H_2O and analysed with HPLC (260 nm, 1 mL/min, MeCN/H_2O, 40/60).

thus meaning that all initially chemically accessible amino groups were acylated. The progress of reactions was monitored by magic angle spinning NMR that was previously demonstrated to be a valuable tool for monitoring the reactions in solid-phase synthesis.[6]

In addition to the very good coupling yields that have been obtained, OMX_{NH2} was shown to be stable toward a variety of conditions used in organic synthesis

[‡] A sample of the dry support (5 g) was packed into a column and equilibrated with 20 mL NaOH 0.5N. The support was then washed with distilled water, until the eluant was neutral. 60 mL of HCl 0.5N were passed through the column and the elute recovered into a beaker. The solution was diluted to 200 mL with water, then 20 mL were titrated with NaOH 0.1N.

[§] The loading, e.g. the number of amino groups on the supports was determined by chemical coupling of Fmoc-L-PheOH directly on the solid support and subsequent cleavage of the Fmoc group (piperidine/DMF, 80/20). The amount of amino groups was determined by reading the absorbance at 290 nm. Loading (mmol/g)= [Abs (290 nm)·V (mL)]/[4950 (ε)·amount (g)].

(chemical reactants and mixing), thus suggesting its general suitability for solid-phase chemistry.

The results obtained in the synthesis of **1** compared favourably to the maximum yield achievable by using $PEGA_{1900}$, a co-polymer of ethylene glycol and acrylamide characterized by swelling properties (it retains about 90% solvent). In this case a maximum yield of 150 $\mu mol/g_{dry\ support}$ was obtained, that corresponds to about 15 $\mu mol/g_{wet\ support}$.

There is an increasing interest in the use of enzymes in solid-phase chemistry.[7–9] However, poor results are often obtained due to limited compatibility of enzyme and solid support. In particular, large enzymes, such as penicillin G acylase (PGA), have been used with only very limited success.[8a,b] To verify that OMX_{NH2} was compatible with enzymatic processes on solid-phase synthesis the enzymatic hydrolysis of the peptide **1** was assayed by using two enzymes previously employed in biocatalysis in polymer-supported synthesis, penicillin G acylase[8] and thermolysin.[7d,9]

Both enzymes are known to be able to hydrolyse PhAc-L-Phe-L-PheOR in solution. However, each one has a specificity for a different amide bond within the molecule. While PGA catalyzes the release of phenylacetic acid (PhAcOH, **2**), thermolysin cleaves the amide bond between the two phenylalanines releasing PhAc-L-PheOH, **3** (Table 1 and Scheme 2).

Both enzymes were clearly active on substrates linked to the OMX_{NH2}, indicating that this support is compatible with enzyme activity. The incomplete conversions are probably related to the limited accessibility of the enzyme to the rigid surface of the support and this was more evident for the larger PGA (88 kDa), than the smaller thermolysin (35 kDa). However, the release of PhAcOH, about 10 $\mu mol/g_{dry}$, was comparable to the yield obtained with $PEGA_{1900}$, which is not fully accessible to PGA. The smaller enzyme thermolysin is able to access to the core of $PEGA_{1900}$ resulting in a near-complete conversion[9] while hydrolysis observed on OMX_{NH2} was significantly lower.

In summary, we show that OMX_{NH2} can be used as an easy to handle and chemically stable support for use in solid-phase chemistry. In addition, OMX_{NH2} is compatible with the use of enzymes. The possibility to vary the size of particles and the high loading of the support, compared to the classical swelling polymers, opens new perspectives for the use of organically modified silicates in solid-phase applications.

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