

Review

SYNTHESIS AND BIOTECHNOLOGICAL APPLICATIONS OF VINYL POLYMER-INORGANIC HYBRID AND MESOPOROUS MATERIALS*

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Abstract We describe the sol-gel synthesis of a new family of organic-inorganic hybrid materials, in which various vinyl polymers are covalently bonded to and uniformly distributed in inorganic oxide matrices. The materials can be tailored to have both good toughness and hardness while maintaining excellent optical transparency. Doping the sol-gel metal oxides with optically active compounds such as *D*-glucose results in new optical rotatory composite materials. Removal of the dopant compounds from the composites affords mesoporous oxide materials, which represents a new, nonsurfactant-templated route to mesoporous molecular sieves. We have successfully immobilized a series of enzymes and other bioactive agents in mesoporous materials. Catalytical activities of the enzyme encapsulated in mesoporous materials were found to be much higher than those encapsulated in microporous materials.

Keywords Vinyl polymer-inorganic hybrid materials, Mesoporous materials, Sol-gel process, Biotech-nological applications

INTRODUCTION

Over the last 10 years or so, we have been working in the general area of sol-gel chemistry. Utilizing our expertise in synthetic polymer chemistry, we have successfully prepared a new family of organic-inorganic hybrid materials, in which organic vinyl polymers, such as polymethacrylates and polystyrene, are covalently and uniformly incorporated into inorganic matrices, such as silica and titania, at the molecular level. As an extension of our work on the hybrid materials, we also doped inorganic sol-gel materials with optically active compounds such as *D*-glucose in an effort to obtain new optical materials and amorphous materials with chiral cavities or imprints. To our surprise, when the compounds such as glucose were removed by extraction, we obtained mesoporous materials with high surface area and pore volume as well as narrow pore size distributions. Thus, we discovered a new, nonsurfactant-templating method to prepared mesoporous molecular sieves. Because of mild reaction conditions in this nonsurfactant-templated synthesis, we were able to encapsulate various enzymes in mesoporous materials and, hence, developed a new technology for enzyme immobilization. In this article, we describe and review the above-mentioned work in three main sections, i.e., 1) vinyl polymer-modified metal oxide sol-gel materials, 2) nonsurfactant-templated synthesis of mesoporous materials and 3) novel immobilization of enzymes and other bioactive substances. Many new results are also presented.

RESULTS AND DISCUSSION

Vinyl Polymer-Modified Metal Oxide Sol-Gel Materials

We have successfully prepared a new class of organic-inorganic hybrid materials, which covalently and uniformly incorporate organic vinyl polymers into inorganic matrices at the molecular level^[1-7]. These hybrid (or

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$$\begin{array}{c} \text{X} \\ \diagup \\ \text{C} \\ \diagdown \\ \text{Y} \end{array} + \begin{array}{c} \text{Z} \\ \diagup \\ \text{C}=\text{C} \\ \diagdown \\ \text{Si(OR)}_m \end{array} \longrightarrow \left(\text{CH}_2 - \begin{array}{c} \text{X} \\ \diagup \\ \text{C} \\ \diagdown \\ \text{Y} \end{array} \right)_x \left(\text{CH}_2 - \begin{array}{c} \text{Z} \\ \diagup \\ \text{C} \\ \diagdown \\ \text{Si(OR)}_m \end{array} \right)_y$$

$$\begin{array}{c} \text{Z} \\ \diagup \\ \text{CH}_2 - \text{C} \\ \diagdown \\ \text{Si(OR)}_m \end{array} + \text{Si(OR)}_4 \xrightarrow[\text{+ H}_2\text{O, -ROH}]{\text{H}^+ / \sim 20^\circ \text{C}} \begin{array}{c} \text{Z} \\ \diagup \\ \text{CH}_2 - \text{C} \\ \diagdown \\ \text{Si(OR)}_m \end{array}$$

$$\begin{array}{c} \text{Z} \\ \diagup \\ \text{CH}_2 - \text{C} \\ \diagdown \\ \text{Si(OR)}_m \end{array} + \text{Si(OH)}_4 \xrightarrow[\text{- H}_2\text{O}]{\text{H}^+} \begin{array}{c} \text{Z} \\ \diagup \\ \text{CH}_2 - \text{C} \\ \diagdown \\ \text{Si} - \text{O} - \text{Si} - \text{O} - \text{Si} - \text{O} - \text{Si} - \text{O} - \text{Si} \\ | \quad \quad \quad | \quad \quad \quad | \quad \quad \quad | \\ \text{O} \quad \quad \quad \text{O} \quad \quad \quad \text{O} \quad \quad \quad \text{O} \end{array}$$

We have also demonstrated that the vinyl polymer components in the inorganic matrices could be further modified to give new hybrid materials or nano-composites^[7, 11]. For example, the poly-acrylonitrile component in the hybrid silica materials undergoes pyrolysis to form carbon fibers of micro- or nano-structures in the silica matrix; the nitrile groups of polyacrylonitrile could also be chemoselectively hydrolyzed to yield new water-soluble polyacrylic modified silica hybrid materials.

The first photochemical synthesis of vinyl polymer-inorganic hybrid materials has been achieved via photoacid-catalyzed sol-gel reactions of metal alkoxides with, e.g. PMMA-MSMA, at various compositions^[12]. The photoacids include diphenyl-iodonium and triphenylsulfonium salts. Furthermore, we have been exploring the technological applications of these new hybrids as dental restorative materials, coatings, ion-selective membranes, catalysts or catalyst supports, reaction templates, etc. The photochemical sol-gel process will be discussed in relation to the development of new lithography and stereolithography technologies for fabrication of coatings and devices with ceramic and/or hybrid materials. We have also developed a new method for the synthesis of the hybrid materials with low volume shrinkage^[13]. Applications of the hybrid materials have been explored, including electronic^[14] and optical^[15] devices as well as dental restorative materials^[16, 17].

Since 1992, the synthesis of mesoporous materials using surfactants as templates has been studied extensively^[18, 19]. These materials might find applications in catalysis, biochemical separation technology and molecular engineering. So far, the surfactants, ionic or neutral, have been the only successful templates for directing the formation of mesoporosity. All the ionic pathways are based on charge matching between the ionic surfactants and ionic inorganic precursors through electrostatic interaction. For the neutral surfactants^[21–23], it was suggested that hydrogen bonding between the surfactants and the precursors directs the formation of mesostructures. We have discovered a new, versatile, low-cost and environmentally-friendly non-surfactant templating route to mesoporous materials^[24, 25]. In an effort to prepare optical rotatory materials^[26], we had

encapsulated optically active organic compounds, such as *D*-glucose, dibenzoyl-*L*-tartaric acid (DBTA), and *D*-maltose, into a silica framework. Upon removing the organic compounds, we discovered that these non-surfactant organic molecules actually act as templates leading to the formation of mesoporous silica materials with large surface areas and pore volumes as well as narrow pore size distributions. The X-ray diffraction (XRD) pattern suggests a disordered channel assembly. The pore diameter can be tuned from ~2 to 6 nanometers simply by varying the template concentration. This non-surfactant pathway provides new opportunities for the preparation of mesostructured materials because of the vast diversity of organic compounds that may function as the templates.

The preparation of colourless, transparent and monolithic disks of the template-containing silicas was achieved by HCl-catalyzed sol-gel reactions of tetraethyl orthosilicate (TEOS) in the presence of the non-surfactant templates, e.g. DBTA, *D*-glucose and *D*-maltose. The mesoporous silica was then obtained upon removing the templates from the template-containing silica in a finely ground powder form by Soxhlet extraction with methanol or water for 2–3 days followed by drying at 373 K overnight. The extent of the template removal was monitored by thermogravimetric analysis (TGA) from the weight loss at 1023 K, at which the organic templates decomposed completely. Brunauer-Emmett-Teller (BET) surface area and pore volume were determined on the samples before and after the extraction by N₂ adsorption-desorption isotherm measurements. The unextracted samples have relatively small BET surface areas and pore volumes. Upon extraction, the surface area and pore volume increased drastically. The surface area was found to correlate linearly with the extent of template removal at various extraction times. For example, the surface area of the sample DB40 containing ~40 percent by weight (%) of DBTA template changed linearly from 2.7 m² g⁻¹ before the extraction to 926 m² g⁻¹ after the template was completely removed by extraction for 3 days.

The BET surface area and pore parameters of the samples after the removal of templates have been measured. In general, an increase in the concentration of the organic templates in the template-containing silica samples results in larger BET surface area and pore volume of the silica powders after the extraction. In the case of DBTA, as the template concentration was increased to 43%, the surface area rose to nearly 1000 m² g⁻¹, beyond which the effect of concentration appeared to stagnate. On the other hand, the pore volume rose continuously from 0.159 cm³ g⁻¹ to 0.998 cm³ g⁻¹ as the concentration of DBTA template was increased from 8 to 57%. The surface area of ~1000 m² g⁻¹ and the pore volume greater than 0.5 cm³ g⁻¹ are among the indications of the formation of mesoporous structures^[18]. It should be noted that both the micropores and mesopores in the materials contribute to the measured surface areas and pore volumes. However, the contributions from the mesopores increase with the template concentration and become dominant at concentrations of ~30%–40% or higher.

The N₂ adsorption-desorption isotherms were determined at various relative pressures (P/P_0) on the silica samples. As examples, the samples (DB40X, DB50X, and DB60X) prepared with high DBTA template concentrations of ~40%, 50% and 60%, respectively, exhibit Type IV isotherms with Type H2 hysteresis loops^[27, 28], whereas the sample (DB20X) prepared with a low template concentration of ~20% shows a completely reversible Type I isotherm, which is typical for microporous structures. The position of the well-defined step in the nitrogen desorption isotherms appears to shift to higher relative pressures as the template concentration is increased. These characteristics are similar to those observed for the surfactant-templated mesoporous materials such as MCM-41, which can be attributed to capillary condensation within narrow tubular mesopores of 3–6 nm in effective diameter. Hence, the materials prepared via the non-surfactant route might possess tubular mesopores like MCM-41.

The pore size and its distribution were also found to be influenced by the concentration of templates. Figure 1 shows pore size distributions. As the template concentration is increased from 43% to 57%, the dominant Barrett-Joyner-Halenda (BJH) pore diameter increases from ~3.4 to 6.0 nm. The fidelity of pore distribution, as indicated by the width at half height of the peak, ranges from 0.3 to 0.8 nm, which is comparable with that observed for the silica mesostructures prepared by the surfactant templating route. The type and size of templates seem to have relatively less effects on the pore size distribution. For example, at approximately the

same template concentration (~50%), the glucose- and maltose-templated mesoporous silicas (i.e. DB50X and MT50X) have almost identical BJH pore size distributions, though the sizes of the two template molecules are very different. The variation in the pore diameter is only 0.4 nm between DB50X and MT50X. These observations differ from those with surfactant templates, for which the pore size depends strongly on the size of surfactants. Hence, the pore size can be varied simply by changing the template concentration. Likewise, the pore volume and BET surface area can be controlled by the template concentration as illustrated in Fig. 1.

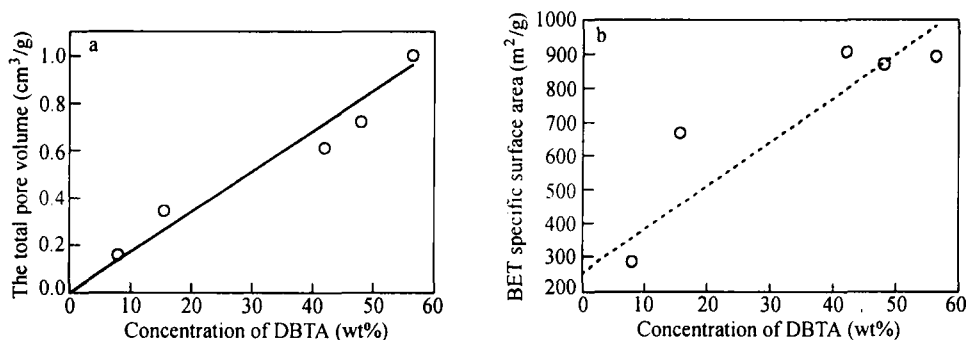


Fig. 1 Effect of the DBTA concentration on a) the total pore volume (cm³/g) and b) BET specific area (m²/g)

The XRD pattern of the mesoporous silica prepared with 48% of maltose, as a typical example, appears to consist of a peak (100 reflection) with a d -spacing of 6.2 nm and a broad, low intensity peak ($d \sim 3.5\text{--}3.1$ nm). Such a pattern is consistent with the mesostructures observed for the silica materials prepared by the surfactant templating routes. With the consideration of thickness of the pore walls, the d -spacing value for the 100 reflection is comparable to the BJH pore diameter (3.2 nm) and the value obtained from transmission electron micrograph. There is a typical amorphous silica halo at a 2θ value of $\sim 23^\circ$ for the non-surfactant templated silica before or after the template removal by extraction, indicating the amorphous nature of the sol-gel silica. The lack of distinct XRD peaks except for the amorphous halo in the materials before the extraction suggests that the template molecules did not crystallize during or after the sol-gel processing. Calcination of the extracted silica at 773 K for 5 h resulted in little change in the amorphous halo but a slight decrease ($\sim 0.2\text{--}0.55$ nm) in the pore diameter, which is common for the silica mesostructures prepared by the sol-gel process. Substantial mesostructures remained even after calcination at 1173 K for 5 h in air.

Though the mechanism of mesophase formation by this non-surfactant pathway has not been understood, we speculate that the aggregation or assembly of the aggregates of the template molecules plays an important role and that the hydrogen bonding between the aggregated templates and the inorganic precursors in solution and/or during the gelation may direct the mesophase formation in a manner similar to the neutral surfactants. The aggregation or assembly should still remain at molecular level without crystallization and have small dimensions, i.e. <400 nm, as evidenced by the facts that the template-containing silica samples exhibit powder XRD patterns of typical amorphous materials and are transparent to visible light. The dimensions of the aggregate or assembly increase with the template concentration, effecting the increase in the mesopore size. However, at very high concentrations (e.g. $>60\%$) crystallization of the templates was observed in several systems. Many other nonsurfactant templates have been investigated. Mesoporous materials including alumina and titania have been synthesized via the nonsurfactant-templating pathway.

Novel Immobilization of Enzymes and Other Bioactive Substances

Immobilization of enzymes is of great importance because of its immense potential for catalytic applications. A popular method is formation of chemical bonding between enzymes and a solid support (we call it “dog on leash”), which often alters the enzymatic activities^[29]. Recently, various enzymes have been entrapped in inorganic oxides such as silica for biocatalysis and biosensor applications through conventional sol-gel process.

However, because of the microporous nature (i.e. pore diameter ≤ 1.5 nm) of conventional sol-gel materials (like a “room with a port-hole”), the catalytic activities of enzymes are hindered by low diffusion rates of substrate molecules and poor accessibility of enzymes inside the materials^[30,31]. We have developed a new, simple method for enzyme immobilization, i.e. encapsulation of enzymes in mesoporous host materials prepared by the nonsurfactant-templating sol-gel route that we developed recently^[26]. Since the pore diameters (e.g. ~ 3.0 nm and tunable, like a “room with a view”) are greater than those in microporous materials, enzymatic activities are significantly increased. Compare to macroporous materials, the interconnecting pore size is small enough to prevent the enzyme from leaking out of its cage. This new method has been demonstrated by encapsulating alkaline phosphatase (ALP), acid phosphatase, lipase and hemoglobin in mesoporous silica^[32].

The encapsulation of ALP was achieved by sol-gel reactions of tetramethyl orthosilicate (TMOS) and tetraethyl orthosilicate (TEOS) in the presence of *D*-glucose as the nonsurfactant template and ALP at room temperature. The TMOS protocol^[31] was modified by introducing *D*-glucose to the system. In the TEOS protocol, HCl-prehydrolyzed TEOS sol was prepared^[24–26]. After addition of *D*-glucose, the sol was neutralized with ammonia, and ALP was then added. As controls, ALP was also encapsulated under identical conditions except for the absence of the glucose template. The samples were characterized with IR, TGA and BET surface analysis before and after removal of the template by water extraction in the same manner as previously reported^[24,25]. General procedures for enzymatic activity assay of ALP were adapted from the literature^[31–33]. After water extraction, the sample containing 0.08 units of ALP was weighed into the assay mixture consisting of 1.0 mmol/L MgCl_2 and 40 mmol/L tris-HCl buffer (pH 8.0) and incubated for 30 min at room temperature. The substrate *p*-nitrophenyl phosphate (8.0 mmol/L) was added to initiate the reaction. Formation of the product *p*-nitrophenol was monitored with UV-vis spectroscopy.

As shown in Fig. 2, the product concentration increases linearly with reaction time. The samples prepared with glucose template have significantly higher specific activities (i.e. slopes), by factor of ~ 2 to 10, than the samples prepared without the template. The activity also increases with the concentration of *D*-glucose. This activity enhancement could be attributed to the high BET surface area (>600 m²/g), pore diameters (3.0–4.0 nm) and volumes (≥ 0.5 cm³/g), which facilitate the diffusion of substrates and products in the host materials. Since the size of ALP enzyme (≥ 5.0 nm) is greater than the pore diameter, the problem of enzyme leaking as encountered in macroporous biogels is much lessened or eliminated. We have found that the specific activities changed little after exhaustive extraction of the samples with water. In fact, lipase entrapped under similar conditions shows high activity but escapes from the host materials readily because of its much smaller size. Both acid phosphatase and hemoglobin have been encapsulated in mesoporous silica without leakage and their bioactivities are retained.

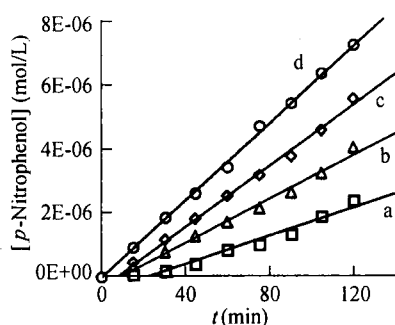


Fig. 2 Plot of the concentration of 4-nitrophenol versus reaction time at pH = 8.0 and 22.5°C under catalysis by ALP encapsulated in porous silicas via the sol-gel reactions of TMOS in the presence of a) 0, b) 38, c) 50 and d) 57 wt% of *D*-glucose

As a summary of this section, we have described a new method for immobilization of enzymes by encapsulating enzymes in mesoporous host materials via sol-gel processing in the presence of *D*-glucose, a

nonsurfactant template. The enzymatic activities are significantly higher than those observed in conventional microporous sol-gel materials. It is noted that most surfactant-templated routes to mesoporous materials involve reagents or conditions that would damage the enzymes. In the nonsurfactant route, *D*-glucose is not only an economical template for mesostructure formation but also biocompatible with ALP and many other enzymes. We have also studied other templates such as tartaric acid derivatives, mono or oligosaccharides, oligopeptides or DNAs, cyclodextrins, glycerol, etc. Immobilization of other enzymes/proteins, such as horseradish peroxidase, glucose oxidase, etc., as well as whole cells and/or organisms, such as red blood cells, yeasts, etc., have also been investigated.

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